



Office de la Propriété
Intellectuelle
du Canada

Un organisme
d'Industrie Canada

Canadian
Intellectual Property
Office

An agency of
Industry Canada

CA 2346942 A1 2000/04/27

(21) 2 346 942

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 1999/10/15
(87) Date publication PCT/PCT Publication Date: 2000/04/27
(85) Entrée phase nationale/National Entry: 2001/04/11
(86) N° demande PCT/PCT Application No.: EP 99/07832
(87) N° publication PCT/PCT Publication No.: WO 00/23584
(30) Priorité/Priority: 1998/10/16 (98119583.7) EP

(51) Cl.Int.⁷/Int.Cl.⁷ C12N 15/12, A61K 38/17, C07K 14/47
(71) Demandeurs/Applicants:
BOEHRINGER INGELHEIM INTERNATIONAL GMBH,
DE;
UNIVERSITY HOSPITAL LEIDEN, NL
(72) Inventeurs/Inventors:
KLADE, CHRISTOPH, AT;
HEIDER, KARL-HEINZ, AT;
AARNOUDSE, CORLIEN A., NL;
SCHRIER, PETER I., NL
(74) Agent: FETHERSTONHAUGH & CO.

(54) Titre : CAMEL, AUTRE PRODUIT DE TRADUCTION DE L'ANTIGENE TUMORAL LAGE-1
(54) Title: CAMEL, AN ALTERNATIVE TRANSLATION PRODUCT OF THE TUMOUR ANTIGEN LAGE-1

(57) Abrégé/Abstract:

The tumor-associated antigen CAMEL and DNA encoding it. The tumor-associated antigen is encoded by an open reading frame of the LAGE-1 gene. The tumor-associated antigen, immunogenic (poly)peptides derived therefrom and DNAs encoding them, are useful for cancer immunotherapy.

BEST AVAILABLE COPY

Canada

<http://opic.gc.ca> • Ottawa-Hull K1A 0C9 • <http://cipo.gc.ca>

OPIC • CIPO 191

OPIC



CIPO

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 15/12, C07K 14/47, A61K 38/17		A1	(11) International Publication Number: WO 00/23584																																																									
			(43) International Publication Date: 27 April 2000 (27.04.00)																																																									
(21) International Application Number: PCT/EP99/07832		(81) Designated States: CA, JP, MX, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).																																																										
(22) International Filing Date: 15 October 1999 (15.10.99)																																																												
(30) Priority Data: 98119583.7 16 October 1998 (16.10.98) EP		Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>																																																										
(71) Applicants (for all designated States except US): BOEHRINGER INGELHEIM INTERNATIONAL GMBH [DE/DE]; Postfach 200, D-55216 Ingelheim am Rhein (DE). UNIVERSITY HOSPITAL LEIDEN [NL/NL]; Rijnsburgerweg 10, NL-2321 RP Leiden (NL).																																																												
(72) Inventors; and																																																												
(75) Inventors/Applicants (for US only): SCHRIER, Peter, I. [NL/NL]; Schelpenkade 17, NL-2313 ZT Leiden (NL). AARNOUDSE, Corlien, A. [NL/NL]; Pr. Alexanderlaan 18, NL-2224 XM Katwijk (NL). HEIDER, Karl-Heinz [DE/AT]; Johann-Strauss-Promenade 4/11, A-2000 Stockerau (AT). KLADE, Christoph [AT/AT]; Gröhmühlgasse 1b/17, A-2700 Wr. Neustadt (AT).																																																												
(74) Agents: LAUDIEN, Dieter; Boehringer Ingelheim GmbH, D-55216 Ingelheim am Rhein (DE) et al.																																																												
(54) Title: CAMEL, AN ALTERNATIVE TRANSLATION PRODUCT OF THE TUMOUR ANTIGEN LAGE-1																																																												
<table border="0"> <tr> <td>CAMEL</td> <td>-----</td> <td></td> </tr> <tr> <td>LAGE-1^S</td> <td>--ATCCTCGTGGGCCCTGACCTTCTCTCTGAGAGCCGGGCAGAGGCTCCG</td> <td>48</td> </tr> <tr> <td>LAGE-1^L</td> <td>GCATCCTCGTGGGCCCTGACCTTCTCTCTGAGAGCCGGGCAGAGGCTCCG</td> <td>50</td> </tr> <tr> <td>NY-ESO-1</td> <td>--ATCCTCGTGGGCCCTGACCTTCTCTCTGAGAGCCGGGCAGAGGCTCCG</td> <td>48</td> </tr> <tr> <td>CAMEL</td> <td>-----CGACGGGCGATGCT</td> <td>14</td> </tr> <tr> <td>LAGE-1^S</td> <td>GAGCCATGCAGGCCGAAGGCCAGGGGCACAGGGGGTTCGACGGGCGATGCT</td> <td>98</td> </tr> <tr> <td>LAGE-1^L</td> <td>GAGCCATGCAGGCCGAAGGCCAGGGGCACAGGGGGTTCGACGGGCGATGCT</td> <td>100</td> </tr> <tr> <td>NY-ESO-1</td> <td>GAGCCATGCAGGCCGAAGGCCAGGGGCACAGGGGGTTCGACGGGCGATGCT</td> <td>98</td> </tr> <tr> <td></td> <td>*****</td> <td></td> </tr> <tr> <td>CAMEL</td> <td>GATGGCCCAGGAGGCCCTGGCATTCTGATGGCCCAGGGGGCAATGCTGG</td> <td>64</td> </tr> <tr> <td>LAGE-1^S</td> <td>GATGGCCCAGGAGGCCCTGGCATTCTGATGGCCCAGGGGGCAATGCTGG</td> <td>148</td> </tr> <tr> <td>LAGE-1^L</td> <td>GATGGCCCAGGAGGCCCTGGCATTCTGATGGCCCAGGGGGCAATGCTGG</td> <td>150</td> </tr> <tr> <td>NY-ESO-1</td> <td>GATGGCCCAGGAGGCCCTGGCATTCTGATGGCCCAGGGGGCAATGCTGG</td> <td>148</td> </tr> <tr> <td></td> <td>*****</td> <td></td> </tr> <tr> <td>CAMEL</td> <td>CGGCCCAGGAGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG</td> <td>114</td> </tr> <tr> <td>LAGE-1^S</td> <td>CGGCCCAGGAGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG</td> <td>198</td> </tr> <tr> <td>LAGE-1^L</td> <td>CGGCCCAGGAGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG</td> <td>200</td> </tr> <tr> <td>LAGE-1^L</td> <td>CGGCCCAGGAGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG</td> <td>198</td> </tr> <tr> <td></td> <td>*****</td> <td></td> </tr> </table>				CAMEL	-----		LAGE-1 ^S	--ATCCTCGTGGGCCCTGACCTTCTCTCTGAGAGCCGGGCAGAGGCTCCG	48	LAGE-1 ^L	GCATCCTCGTGGGCCCTGACCTTCTCTCTGAGAGCCGGGCAGAGGCTCCG	50	NY-ESO-1	--ATCCTCGTGGGCCCTGACCTTCTCTCTGAGAGCCGGGCAGAGGCTCCG	48	CAMEL	-----CGACGGGCGATGCT	14	LAGE-1 ^S	GAGCCATGCAGGCCGAAGGCCAGGGGCACAGGGGGTTCGACGGGCGATGCT	98	LAGE-1 ^L	GAGCCATGCAGGCCGAAGGCCAGGGGCACAGGGGGTTCGACGGGCGATGCT	100	NY-ESO-1	GAGCCATGCAGGCCGAAGGCCAGGGGCACAGGGGGTTCGACGGGCGATGCT	98		*****		CAMEL	GATGGCCCAGGAGGCCCTGGCATTCTGATGGCCCAGGGGGCAATGCTGG	64	LAGE-1 ^S	GATGGCCCAGGAGGCCCTGGCATTCTGATGGCCCAGGGGGCAATGCTGG	148	LAGE-1 ^L	GATGGCCCAGGAGGCCCTGGCATTCTGATGGCCCAGGGGGCAATGCTGG	150	NY-ESO-1	GATGGCCCAGGAGGCCCTGGCATTCTGATGGCCCAGGGGGCAATGCTGG	148		*****		CAMEL	CGGCCCAGGAGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG	114	LAGE-1 ^S	CGGCCCAGGAGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG	198	LAGE-1 ^L	CGGCCCAGGAGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG	200	LAGE-1 ^L	CGGCCCAGGAGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG	198		*****	
CAMEL	-----																																																											
LAGE-1 ^S	--ATCCTCGTGGGCCCTGACCTTCTCTCTGAGAGCCGGGCAGAGGCTCCG	48																																																										
LAGE-1 ^L	GCATCCTCGTGGGCCCTGACCTTCTCTCTGAGAGCCGGGCAGAGGCTCCG	50																																																										
NY-ESO-1	--ATCCTCGTGGGCCCTGACCTTCTCTCTGAGAGCCGGGCAGAGGCTCCG	48																																																										
CAMEL	-----CGACGGGCGATGCT	14																																																										
LAGE-1 ^S	GAGCCATGCAGGCCGAAGGCCAGGGGCACAGGGGGTTCGACGGGCGATGCT	98																																																										
LAGE-1 ^L	GAGCCATGCAGGCCGAAGGCCAGGGGCACAGGGGGTTCGACGGGCGATGCT	100																																																										
NY-ESO-1	GAGCCATGCAGGCCGAAGGCCAGGGGCACAGGGGGTTCGACGGGCGATGCT	98																																																										

CAMEL	GATGGCCCAGGAGGCCCTGGCATTCTGATGGCCCAGGGGGCAATGCTGG	64																																																										
LAGE-1 ^S	GATGGCCCAGGAGGCCCTGGCATTCTGATGGCCCAGGGGGCAATGCTGG	148																																																										
LAGE-1 ^L	GATGGCCCAGGAGGCCCTGGCATTCTGATGGCCCAGGGGGCAATGCTGG	150																																																										
NY-ESO-1	GATGGCCCAGGAGGCCCTGGCATTCTGATGGCCCAGGGGGCAATGCTGG	148																																																										

CAMEL	CGGCCCAGGAGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG	114																																																										
LAGE-1 ^S	CGGCCCAGGAGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG	198																																																										
LAGE-1 ^L	CGGCCCAGGAGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG	200																																																										
LAGE-1 ^L	CGGCCCAGGAGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG	198																																																										

(57) Abstract																																																												
<p>The tumor-associated antigen CAMEL and DNA encoding it. The tumor-associated antigen is encoded by an open reading frame of the LAGE-1 gene. The tumor-associated antigen, immunogenic (poly)peptides derived therefrom and DNAs encoding them, are useful for cancer immunotherapy.</p>																																																												

CAMEL, AN ALTERNATIVE TRANSLATION PRODUCT OF THE TUMOUR ANTIGEN LAGE-1

The present invention relates to the field of cancer therapy, more specifically to tumor-associated antigens.

Cytotoxic T lymphocytes (CTLs) play an important role in the defense
5 against melanoma. Melanoma-specific CTL clones have been obtained from either tumor infiltrating lymphocytes (TIL) *in vitro* stimulated with cytokines, or from peripheral blood mononuclear cells (PBMC) cultured with (autologous) tumor cells. T cell responses against tumor cells are enhanced by cytokine transfection of the tumor cells, both in animal models and in *in*
10 *vitro* human culture systems. (van Elsas et al., 1997; Gansbacher et al., 1990; Tepper et al., 1989; Fearon et al., 1990; Dranoff et al., 1993)

The antigens recognized by the tumor-specific T cells become better defined by the development of molecular cloning techniques. These T cell targets can be divided in three groups: 1) tumor-specific antigens, not
15 expressed in healthy tissues, except testis and placenta (e.g., MAGE, BAGE, GAGE, NY-ESO-1, LAGE-1); 2) antigens that are lineage-specific and expressed in both melanoma and melanocytes (e.g., MART-1/ Melan-A, gp100, tyrosinase) and 3) unique, mutated antigens (e.g., β -catenin, CDK4, MUM-1) (reviewed by Van den Eynde and Brichard, 1995).

20 By means of Representational Difference Analysis (RDA), a PCR-based method that has been used to identify genes with tissue-specific or tumor-specific expression, the LAGE-1 and NY-ESO1 genes were identified as being tumor specific by screening cDNA libraries from melanoma cell lines with a primer from a cDNA clone enriched in melanoma-specific sequences
25 (Lethe et al., 1998).

NY-ESO-1 is a gene originally identified by SEREX technology (Chen et al., 1997). It was shown to have two different reading frames (DNA sequences and derived protein sequences given in SEQ ID NO: 7 - 10), translation

products of which were shown to contain epitopes of tumor specific T-cells (Jäger et al., 1998; Wang et al., 1998).

It was an object of the present invention to provide a novel tumor-associated antigen.

- 5 To solve the problem underlying the invention, melanoma cell line 518A2 and its IL-2- or GM-CSF-transfectants were compared for their CTL stimulating capacity *in vitro*. Stimulation of autologous PBMC with the IL-2 producing melanoma cells resulted in a melanoma-specific CTL response (van Elsas et al., 1997). CTL clones derived from this culture recognized,
10 besides autologous melanoma cell lines, also a panel of HLA-A*0201 positive melanoma cell lines, but were not reactive with normal melanocytes. Although 518A2 was shown to express a number of antigens previously identified to be recognized by anti-melanoma CTL (van Elsas et al., 1996), the CTL clones available recognize a new melanoma-specific
15 antigen that is immunodominant in 518A2.

- In the experiments of the present invention, the target structure that was recognized by one of these CTL clones was fully characterized and named CAMEL (CTL-recognized Antigen on Melanoma). These sequences are described in the attached sequence listing as SEQ ID NO: 1 and SEQ ID
20 NO: 2.

It was surprisingly found that CAMEL is encoded by a reading frame of the LAGE-1^s-cDNA (SEQ ID NO:3) that is distinct from that encoding the putative LAGE-1 protein (SEQ ID NO: 4). (This reading frame is designated ORF-1.)

- 25 In the present invention, a cDNA clone was identified that lacks the first 84 bp of the LAGE-1^s sequence (SEQ ID NO: 3) which means that it is devoid of the initiation codon at position 54 of that sequence (Fig. 2a). The first possible translation initiation site in this clone (4H8) is the ATG at position 94 of LAGE-1^s (SEQ ID NO: 3), which is however, not in frame with

the first ATG at position 54. Therefore, the CAMEL protein (SEQ ID NO: 2) translated from the 4H8 cDNA clone is different from the putative LAGE-1^S protein (SEQ ID NO: 4).

5 In a first aspect, the present invention is directed to the tumor-associated antigen CAMEL (SEQ ID NO: 2) which is encoded by an isolated DNA molecule with the sequence as defined in SEQ ID NO: 1.

The coding sequence of CAMEL corresponds to the ORF-1 of LAGE-1 cDNA (Lethe et al., 1997; WO 98/32855).

10 In the present invention „ORF-1“ is defined as the open reading frame starting with ATG at position 94 of SEQ ID NO:3 (LAGE-1^S), which corresponds to position 10 in SEQ ID NO: 1 (CAMEL), to position 96 in SEQ ID NO: 5 (LAGE-1^L)

15 In a further aspect, the present invention relates to immunogenic (poly)peptides derived from CAMEL. A first group of peptides is selected from peptides inducing a humoral immune response (induction of antibodies). Such peptides are selected by randomly choosing continuous stretches of amino acids (at least 12-15), applying them to an individual and confirming the generation of antibodies by standard immunological assays, e.g. ELISA. This group of immunogenic (poly)peptides also encompasses
20 the entire CAMEL antigen or larger fragments thereof.

The second group of peptides, which is preferred, can be presented by MHC molecules (in humans: HLA molecules), they have the potential to induce an immune response, in particular by eliciting a CTL response.

25 In a preferred embodiment, immunogenic peptides which have the ability to elicit a CTL response, are selected from peptides with the sequence set forth in SEQ ID NO: 11, 12, 24, 25 and 26.

To obtain peptides that have the ability to elicit a cellular immune response, the selection of peptide sequences from a given antigen is, in the first place, based on the requirement for such peptide to bind to an MHC molecule present in the repertoire of the patient to be treated. Two classes of MHC molecules are distinguished, class I and class II. Class I molecules consist of a membrane-inserted heavy chain and a non-covalently attached light chain. In their structure, MHC class I molecules resemble a moose's head, the antlers forming a groove which is recognized by the peptide. In humans, HLA-A, B and C are the "classical" MHC class I molecules.

- 5
10 Additional immunogenic peptides may be identified by methods known in the art which rely on the correlation between MHC-binding and CTL induction, e.g. those used by Stauss et al., 1992, who identified candidate T-cell epitopes in human papilloma virus.

- 15 Since immunogenic peptides can be predicted based on their "peptide binding motif" synthetic peptides which represent CTL epitopes may be designed and synthesized. Several methods, which are useful in the present invention for designing peptides, have been used to identify CTL epitopes from known protein antigens.

- 20 It is well established that every MHC class I allelic product has allele-specific requirements for the peptide ligand that binds to its groove and that it ultimately presents. These requirements were summarized as a motif by Falk et al., 1991. A large number of MHC peptide motifs and MHC ligands have become known to date. A method to search a known protein sequence for epitopes fitting to a given class I molecule, which is based on this knowledge and which may be used in the present invention, was reviewed by Rammensee et al., 1995. It comprises the following steps: first, the protein sequence is screened for stretches fitting to the basic anchor motif (two anchors in most cases), whereby allowance should be made for some variation in peptide lengths as well as in anchor occupancy.
- 25
30 If a motif, for example calls for 9mers with Ile or Leu at the end, 10mers with

a fitting C-terminus should be considered as well, and other aliphatic residues at the C-terminus, like Val or Met, should also be considered. In this way, a list of peptide candidates is obtained. These are inspected for having as many non-anchor residues as possible in common with ligand already known, or with the residues listed among the "preferred residues" or "others" on top of each motif (Table, given by Rammensee et al., 1995), for various HLA molecules. Binding assays can be performed at this stage to exclude weak binders which occur frequently among peptides conforming to a basic motif. If a detailed study on peptide binding requirements is available, the candidates can also be screened for non-anchor residues detrimental or optimal for binding (Ruppert et al., 1993). One should keep in mind, however, that pure peptide binding motifs can be misleading in the search for natural ligands, since other constraints, such as enzyme specificity during antigen processing and specificity of transporters or chaperones, are likely to contribute to ligand identity in addition to the MHC binding specificity.

This approach was successfully applied by, inter alia, Kawakami et al., 1995, to identify gp100 epitopes based on known HLA-A2.1 motifs. The validity of the method was confirmed by identifying, in parallel, the epitope regions by using COS cells transfected with cDNA fragments generated by sequential deletion and testing for T-cell reactivity, as described above.

Recently, data bases and prediction algorithms have become available that enable to predict, with great reliability, peptide epitopes that bind to HLA molecules of interest.

Examples for peptide candidates with potential immunogenicity that can be derived from the tumor-associated antigen of the present invention, are the CAMEL-derived peptides with the sequence HLSPDQGRE and LMAQEALAE for HLA-A3 or RMAVPLLRR for HLA-A3101. Similarly, other peptides for these or for other alleles can be determined by the method mentioned above.

The peptide binding can be tested in peptide binding assays. In order to determine the immunogenicity of the selected peptide or peptide equivalent, as defined below, which is the crucial parameter for peptide-based vaccine development and which in most cases strongly correlates with the stability of the peptide-MHC interaction (van der Burg et al., 1996), the methods described by Sette et al., 1994, in combination with quantitative HLA-binding assays, may be used. Alternatively, immunogenicity of the selected peptide may be checked by performing *in vitro* CTL induction by known methods e.g. as described below for *ex vivo* CTL induction.

- 10 Alternatively to peptides derived from the naturally expressed tumor antigens, functional equivalents thereof, i.e. peptides with partially altered sequences or substances mimicking peptides, e.g. "peptidomimetics" or retro-inverso peptides, may be obtained by the following methods:

15 To enhance the immunogenicity of the peptides, amino acid substitutions may be introduced at anchor positions to increase peptide MHC class I-binding affinity. The modified peptides are subsequently evaluated for enhanced binding and immunogenicity by screening for recognition by TIL (tumor-infiltrating lymphocytes) and CTL induction as described by Parkhurst et al., 1996, and Bakker et al., 1997.

- 20 Another method useful in the present invention to find more immunogenic peptides by screening peptide libraries with a known CTL was described by Blake et al. 1996; it suggests the use of combinatorial peptide libraries for constructing functional mimics of tumor epitopes recognized by MHC class I-restricted CTLs.

- 25 In principle, the selection of peptides capable of eliciting a cellular immune response is carried out in several steps, as described in WO 97/30721, which disclosure is incorporated herein by reference. In short, the candidates are first tested for their binding ability to an MHC molecule; subsequently good binders are tested for immunogenicity. A general

strategy for obtaining efficient immunogenic peptides has been described by Schweighoffer, 1997.

The polypeptide of the present invention or immunogenic peptides derived from its sequence, respectively, can be produced recombinantly or by peptide synthesis, as described in WO 96/10413, the disclosure of which is incorporated herein by reference. For recombinant production, a DNA molecule encoding the antigen or the CTL peptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell, cultivated under conditions suitable for expression, recovered and purified. For chemical synthesis, various conventional techniques may be used, e.g. commercially available automatic synthesizers.

The tumor antigen of the present invention and the immunogenic peptides derived therefrom or the respective peptide equivalents are useful in cancer therapy, e.g. to induce, in the context of the appropriate MHC presenting molecule, an immunological response to tumors which express the corresponding antigen determinants. The induction of CTLs can be accomplished *in vivo* or *ex vivo*.

For *in vivo* induction of CTLs, a pharmaceutical composition comprising the peptide/antigen is administered to an individual suffering from a tumor associated with the respective tumor antigen in an amount sufficient to elicit an effective CTL response to the antigen-bearing tumor. Thus, the present invention provides pharmaceutical compositions for therapeutic treatment which are intended for parenteral, topical, oral or local administration. Preferably, the compositions are for parenteral administration, e.g. for intravenous, subcutaneous, intradermal or intramuscular application. The peptides/antigens are dissolved or suspended in a pharmaceutically acceptable carrier, preferably an aqueous carrier. The composition may contain additional auxiliary substances, e.g. buffering agents, etc. The peptides may be used alone or in combination with adjuvants, e.g. saponins, alum, or, in a particularly preferred embodiment, polyocations,

like polyarginine or polylysine. The peptides may also be linked to components assisting CTL priming, e.g. T helper peptides, lipids or liposomes or coadministered with such components or with immunostimulating substances, e.g. cytokines (IL-2, IFN- γ). Methods and
5 compositions for preparing and administering pharmaceutical compositions for therapeutic treatments are described in WO 95/04542 and WO 97/30721 the disclosures of which are herein incorporated by reference.

The immunogenic peptides may also be used to elicit a CTL response
10 *ex vivo*. An *ex vivo* CTL response to a tumor expressing the antigen is induced by incubating a patient's CTL precursor cells together with antigen presenting cells and the immunogenic peptide. The thus activated CTLs are allowed to mature and expand to effector CTLs which are then readministered to the patient. Alternatively, the tumor antigen may be
15 pulsed onto APCs which present MHC class II-reactive peptides (Mayordomo et al., 1995; Zitvogel et al., 1996). A suitable method for loading peptides onto cells, e.g. dendritic cells, is disclosed in WO 97/19169.

The peptides of the invention are preferably applied as a combination of
20 peptides, e.g. different CAMEL-peptides. In an even more preferred embodiment, the peptides of the invention are combined with peptides derived from other tumor antigens, e.g. LAGE-1 and ESO-NY-1. The selection of the peptides is optimized towards covering multiple HLA types in order to be useful for a broad population of patients and/or towards a
25 broad variety of malignancies, which is taken into account by combining peptides from a large variety of tumor antigens. The number of peptides suitable to be combined to yield an efficient therapy may vary within a broad range, e.g. from about 2 to approximately 100.

In a further aspect, the present invention is directed to an isolated DNA
30 molecule with the sequence set forth in SEQ ID NO: 1 encoding CAMEL.

This DNA molecule, which is designated "CAMEL-DNA", contains the ORF-1 of LAGE-1 cDNA which is defined by nucleotides 54 - 336 of the sequence set forth in SEQ ID NO: 3.

The CAMEL-DNA of the present invention, or the corresponding RNA, may
5 be used, as an alternative to the use of the protein or the peptide, for cancer immunotherapy. Alternatively to using the natural sequence or fragments thereof, engineered derivatives may be utilized. These include sequences modified to encode (poly)peptides with improved immunogenicity, e.g. taking into account the modifications described above
10 for the peptides. Another form of modification is the assembly of multiple sequences encoding immunologically relevant peptides in a so-called string-of-beads fashion, as described by Toes et al., 1997. The sequences may also be modified by adding auxiliary coding elements, e.g. targeting functions that ensure more efficient delivery and processing of the
15 immunogen (e.g. Wu et al., 1995).

The nucleic acid molecules may be delivered either directly or as part of a recombinant virus or bacterium. In principle, any method that is known for gene therapy may be applied for nucleic acid-based cancer immunotherapy, both *in vivo* and *ex vivo*.

20 Examples for *in vivo* delivery are direct injection (injection of "naked" DNA) either intramuscularly or by "gene gun", which has been shown to result in the generation of CTLs against tumor antigens. Examples for recombinant organisms are vaccinia virus, fowlpox virus and adenovirus or Listeria monocytogenes (see Coulie, 1997 for a comprehensive review).
25 Furthermore, synthetic nucleic acid carriers like cationic lipids, microspheres, microbeads, liposomes may be useful for *in vivo* delivery of the sequence encoding respective antigen/peptide. Similarly as for peptides, various auxiliary agents that enhance the immune response may be co-applied, e.g. cytokines, either as proteins or as plasmids encoding
30 these.

Examples for *ex vivo* delivery are transfection of dendritic cells (Tuting, T., 1997) or other antigen presenting cells which are applied as a cellular cancer vaccine.

The present invention is also directed to the use of cells that express the
5 tumor-associated antigen of the invention, either naturally or upon transfection with the respective coding sequence, for the preparation of a tumor vaccine.

In the present invention, it has been shown that CTL clones raised against IL-2 producing melanoma cell line 518/IL-2.14 are reactive against two
10 alternatively spliced variants of LAGE-1, LAGE-1^S (SEQ ID NO: 3) and LAGE-1^L (SEQ ID NO: 5) and NY-ESO-1 (SEQ ID NO: 9). NY-ESO-1 is a recently described tumor antigen, identified by screening a cDNA library of an esophagus carcinoma with autologous patient serum (SEREX-method (Chen et al., 1997)). NY-ESO-1 is expressed in different tumor types but
15 not in healthy tissues except the testis.

In the present invention, the epitope of specific CTL 1/29 was determined by cDNA expression cloning and a truncated LAGE-1 cDNA clone was found. This truncation led to the identification of the peptide epitope in an
20 alternative reading frame, since the "normal" translation initiation site of LAGE-1 was absent. However, COS/HLA-A*0201 cells transfected with full length LAGE-1 or NY-ESO-1 cDNA clones could stimulate the CTL clone to TNF- α production as well. This probably means that two different proteins can be translated from one single mRNA.

NY-ESO-1 also has been described as the target of melanoma-specific
25 HLA-A*0201 restricted CTL clones, which recognize an epitope translated in ORF3, located between aa 155 and 167 (Jäger et al., 1998). Therefore, it is very likely that also LAGE-1^S will be recognized by these clones, but not LAGE-1^L, since the protein sequence is different at this part of the molecule. The CAMEL-specific CTL clones recognize a peptide in an

alternative reading frame, which is encoded in both LAGE-1 and NY-ESO-1. This means that tumor cells expressing either LAGE-1 or NY-ESO-1 can be recognized by MLMAQEALAFI-specific CTL, which might enlarge the number of tumors that can be treated with immunotherapy based on this peptide.

Brief description of the Figures:

Figure 1: COS-7 transfection experiments with cDNA clone CAMEL and deletion constructs

- a) COS-7 cells were transfected with cDNAs as indicated and tested with CTL 1/29 in a TNF- α release assay.
- b) Deletion constructs of CAMEL cDNA were cotransfected with HLA-A*0201 cDNA in COS-7, followed by a TNF- α release assay with CTL 1/29. The PCR clones contain the numbers of nucleotides of the CAMEL cDNA as indicated.

Figure 2:

- a) Nucleotide alignment of cDNA clones CAMEL, LAGE-1^S, LAGE-1^L and NY-ESO-1.
- b) Protein translations of the cDNA clones LAGE-1^S, LAGE-1^L and NY-ESO-1. The translation of CAMEL is identical to the translation of LAGE-1^{SL} in ORF1. Although ORF3 seems the most putative one, the CTL epitope is encoded in ORF1 (underlined).

Figure 3: Characterisation of peptides recognized by CTL clone 1/29

- a) TNF- α release assay with predicted HLA-A*0201 binding CAMEL peptides. Peptides as indicated were loaded on BLM, an

HLA-A*0201⁺ melanoma cell line, at a concentration of 10 µg/ml and tested with CTL 1/29 in a TNF-α release assay.

- b) The effects of increasing concentrations of peptides, derived from the major target epitope MLMAQEALAFI on recognition by CTL 1/29.
- 5 Various concentrations of peptides as indicated were loaded on HLA-A*0201⁺ cells and tested in a TNF-α release assay with CTL 1/29.

Figure 4: LAGE-1^{SL} and NY-ESO-1 encode the CTL epitope

COS/HLA-A*0201 cells were transfected with these cDNA clones and

10 reactivity with CTL 1/29 was measured in a TNF-α release assay.

Figure 5: His-tagged CAMEL protein, synthesized in E.coli

Figure 6: Expression of LAGE-1^{SL} and NY-ESO-1 in healthy human tissues and melanoma cell lines

- a) Northern Blot analysis of the expression of LAGE-1/NY-ESO-1 in a
- 15 panel of healthy human tissues as indicated. The Blot was hybridised with ³²P-dCTP-labeled LAGE-1^S cDNA.
- b) RT-PCR for LAGE-1 and NY-ESO-1. To discriminate between
- LAGE-1 and NY-ESO-1 mRNA, the same panel of melanoma cell
- lines was analysed by RT-PCR with gene-specific primers. Melanoma
- 20 cell lines as indicated were used as targets in a TNF-α release assay with CTL 1/29.

Figure 7: Immunohistochemical analysis of CAMEL expression in human tumors

Figure 8: Stabilization of HLA-A2 surface expression by synthetic

25 peptides on T2-cells

Brief description of the sequences:

- SEQ ID NO: 1: CAMEL (4H8) cDNA sequence and translation
- SEQ ID NO: 2: CAMEL protein sequence
- 5 SEQ ID NO: 3: LAGE-1^S cDNA sequence and translation
- SEQ ID NO: 4: LAGE-1^S protein sequence
- SEQ ID NO: 5: LAGE-1^L cDNA sequence and translation
- SEQ ID NO: 6: LAGE-1^L protein sequence
- SEQ ID NO: 7: NY-ESO-1 cDNA sequence and translation
- 10 SEQ ID NO: 8: NY-ESO-1 protein sequence
- SEQ ID NO: 9: NY-ESO-1 cDNA and alternative translation
- SEQ ID NO: 10: protein sequence of alternatively translated NY-ESO-1
- SEQ ID NO: 11: peptide sequence of the CAMEL CTL epitope (11-mer)
- SEQ ID NO: 12: peptide sequence of the CAMEL CTL epitope (10-mer)
- 15 SEQ ID NO: 13: oligonucleotide SP6F-pSV
- SEQ ID NO: 14: oligonucleotide R1
- SEQ ID NO: 15: oligonucleotide R2
- SEQ ID NO: 16: oligonucleotide T7R-pSV
- SEQ ID NO: 17: oligonucleotide F3
- 20 SEQ ID NO: 18: oligonucleotide ESO-1B
- SEQ ID NO: 19: oligonucleotide ESO-1A
- SEQ ID NO: 20: oligonucleotide 4H8-A
- SEQ ID NO: 21: oligonucleotide 4H8-C
- SEQ ID NO: 22: oligonucleotide CAMEL-XHO
- 25 SEQ ID NO: 23: oligonucleotide CAMEL-KPN

WO 00/23584

PCT/EP99/07832

14

- SEQ ID NO: 24 peptide CAMEL10
SEQ ID NO: 25 peptide CAMEL16
SEQ ID NO: 26 peptide CAMEL17
SEQ ID NO: 27 tyrosinase peptide
5 SEQ ID NO: 28 MAGE-3 peptide

In the Examples, if not stated otherwise, the following materials and methods were used

a) Cell cultures

- 10 Melanoma cell lines and COS-7 cells were maintained in DMEM containing 4.5 mM glucose supplemented with 8% FCS, 2 mM L-glutamine, 100 µg/ml of each penicillin and streptomycin. Melanoma cell line 518A2 was established from the dissected metastasis of a male patient in 1985, as described before (Versteeg et al., 1988). An IL-2 producing variant, 518/IL-
15 2.14, was obtained by transfection of 518A2 with the IL-2 cDNA (Osanto et al., 1993). Other melanoma cells that were used as targets in TNF-α release assay are FM3.29, FM6, FM28.4 and FM55P (gifts from J. Zeuthen, Denmark), MM127, MM415, MM485 (gifts from N. Hayward, Australia), SK-MEL-23, SK-MEL-29 (obtained from T. Wölfel, Mainz), Mi10221, Mi3046/2,
20 NA8, BLM (obtained from M. Visseren, Leiden). EBV-transformed B-LCL and the TNF-α-sensitive WEHI-164 clone 13 (a gift from Dr. P. Coulie, Brussels) were cultured in RPMI-1640, supplemented with L-glutamine and antibiotics as above, and 10% FCS.

- With the IL-2 producing cell line 518/IL-2.14 and autologous peripheral
25 blood mononuclear cells (PBMC) a CTL induction was performed, resulting in melanoma-specific HLA-A*0201 restricted CTL clones (van Elsas et al., 1997). The identification of the epitope of one of these clones, CTL 1/29, is reported here.

b) cDNA expression cloning

A cDNA library of 518/IL-2.14 was constructed in the expression vector pSVsport1 (GIBCO, BRL) using the Superscript Plasmid System (GIBCO, BRL). As to that purpose, poly-A⁺ mRNA was isolated using the Fast-Track system (Invitrogen), followed by reverse-transcription with an oligo-dT/NotI primer. Sall adapters were ligated to ds-cDNA and after NotI digestion and size fractionation, cDNA fragments were cloned into the pSVsport1 vector digested with Sall and NotI. After electroporation into ElectroMAX-DH10B (GIBCO, BRL) (following the manufacturers instructions) and selection for ampicillin resistance, 50-100 colonies were pooled for mini DNA isolation (QIAprep 8 plasmid kit, Qiagen). The in this way obtained cDNA pools were transfected in duplicate into COS-7 cells, together with the restriction element HLA-A*0201 (pBJ1.neo/HLA-A*0201, (Lin et al., 1990)), using the DEAE-dextran method. Briefly, COS-7 cells were seeded in 96-wells flatbottom plates at 1.5×10^4 cells per well in 100 μ l DMEM, 8% FCS. After 2 hours, medium was replaced by 30 μ l transfection mixture, containing 100 ng cDNA pool, 100 ng HLA-A*0201 cDNA, 400 ng/ml DEAE-dextran and 100 μ M chloroquine in serum free DMEM. Cells were incubated for 4 hours at 37°C and shocked for 2 minutes by the addition of 50 μ l 10% DMSO in PBS. The shock medium was replaced by 200 μ l DMEM, 8% FCS, and 48 hours later the cells were used as target cells for CTL in a TNF- α release assay.

c) Deletion constructs

Deletion constructs of cDNA clone 4H8 were obtained by PCR. PCR products were cloned in vector pCR3.uni (TA cloning system, Invitrogen). The constructs pCR-246 and pCR-464 were made with the vector-based forward primer, SP6F-pSV (SEQ ID NO: 13) and the reverse primers in cDNA 4H8, R1 (SEQ ID NO: 14) and R2 (SEQ ID NO: 15) respectively. As a control the complete 679 bp cDNA was cloned by PCR with two primers

on the pSVsport vector, SP6F-pSV (SEQ ID NO: 13) and T7R-pSV (SEQ ID NO: 16), resulting in pCR-679.

d) TNF- α release assay

CTL reactivity against tumor target cells, transfected COS-7 or peptide
5 loaded cells was measured in a TNF- α release assay. Target cells were seeded in duplicate or triplicate at 1.5×10^4 cells per well in a 96-wells flat bottom plate and 1500-2000 CTL were added to each well, in a total volume of 100 μ l / well (IMDM, supplemented with antibiotics and 5% FCS). After 24 hours of co-culturing of effector and target cells, 50 μ l out of each
10 well was added to a fresh 96-wells flatbottom plate, containing 50 μ l (4.5×10^4) TNF- α -sensitive WEHI-164 cells per well in IMDM, supplemented with antibiotics, 5% FCS, 2 μ g/ml Actinomycin D and 40 mM LiCl. A viability staining was performed 24 hours later by the addition of 50 μ l of
15 3-(4,5dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) solution (2.5 mg/ml in PBS). After incubation for 2-4 hours at 37°C the OD₅₅₀₋₆₅₀ was measured. TNF- α release in pg/ml was calculated from a standard with known TNF- α concentrations.

e) Northern Blot analysis

To determine expression in healthy tissues a Multiple Tissue Northern Blot
20 was obtained commercially (Clontech). As a probe, LAGE-1 cDNA was used, labeled with γ -³²P-dCTP by use of the Mega-Prime Labeling kit (Amersham).

f) RT-PCR

cDNA synthesis was performed using oligo-dT and M-MLV reverse
25 transcriptase (Promega). Primers used for LAGE-1 specific PCR were the F3 (SEQ ID NO: 17) and ESO-1B primer (SEQ ID NO: 18). ESO-1B was also used as a reverse primer in the NY-ESO-1-specific PCR, while ESO-1A (SEQ ID NO: 19) was the forward primer in this reaction (Chen et

al., 1997). Reactions were performed in a Biometra-Uno or -Trio programmed as follows: 5 minutes 95°C, 30 cycles of 1 min. 95°C, 1 min. 58°C, 1 min. 72°C, followed by 10 minutes 72°C.

g) Expression of CAMEL in E. Coli

- 5 A fragment containing the coding sequence of CAMEL was made by PCR with the following primers:

4H8-A: GAAGAACATATGCTGATGGCCCAGGAGGC (SEQ ID NO: 20)

4H8-C: TTAAAGATCTCAGAACCGCCCCTGGTCG (SEQ ID NO: 21)

- This fragment was digested with NdeI and BglII and cloned in the NdeI and
10 BamHI sites of vector pET19b (Novagen, Madison, WI). This vector contains a 6xHis-tag coding sequence, allowing detection of the His-tagged protein with an anti-His antibody. The pET19b-CAMEL construct was transformed into BL21(DE3)pLysS E. coli bacteria (Novagen, Madison, WI). After culturing the bacteria at 30°C until an OD₆₀₀ = 0.5, IPTG (1 mM) was
15 added to induce overexpression of the His-tagged CAMEL protein. Samples were taken at 0h and 4h after IPTG induction and lysates of these samples were tested on a Western Blot with the Penta-His Antibody (Qiagen) according to the Western and Colony Blot protocol of the supplier. The His-tagged protein was visualized using the SuperSignal Substrate
20 system for Western blotting (Pierce, Rockford, US).

h) Preparation of anti-CAMEL antisera

Antibodies against the CAMEL protein were raised by immunizing two rabbits with three synthetic peptides derived from hydrophobic regions of this molecule :

- 25 F4: (K)GAMLAQERRVPRAAEV(K) (pos. 15-31 of SEQ ID NO: 2)
A5: (K)GQQGPRGREEAPRGVRM(K) (pos. 36 -52 of SEQ ID NO: 2)
B5: (K)KRRMEGAPAGPGGRTAA(K) (pos. 58 -73 of SEQ ID NO: 2)

(The lysine residues at both termini enable the peptides to be linked to KLH.)

For rabbit no. 1, 1 mg of each peptide was linked chemically to 2.5 mg of the carrier molecule KLH (Keyhole Limpet Hemocyanin) and after dialyzing, 0.8 mg of this protein in CFA (Complete Freund's Adjuvants) was weekly injected subcutaneously. Another rabbit (no. 2) was injected six times with the three peptides, not linked to KLH, following the scheme of 300 µg s.c. in CFA, 300 µg s.c. in IFA, 4x boost of 150 µg. The reactivity and specificity of the antisera were confirmed in ELISA and Western blot experiments. After four immunizations, antisera of both rabbits were reactive with the recombinant CAMEL protein synthesized in *E.coli*, but differed in their precise epitope: rabbit no. 1 produced antibodies against the CAMEL-B5 peptide, whereas the serum of rabbit no. 2 reacted with peptide F4. The antisera will further be referred to as "antiserum B5" and "antiserum F4".

j) Preparation of CAMEL-EGFP fusion proteins

The CAMEL coding sequence was fused to the *Aequorea victoria* -derived Green Fluorescent Protein (GFP). The CAMEL cDNA molecule was cloned into the pEGFP-N1 vector (Clontech), which contains a cDNA encoding the Enhanced, red shifted variant of GFP. In order to clone the cDNA molecule in frame with the EGFP cDNA and unidirectional, two primers were designed. The forward primer designated CAMEL-XHO (TTACTCGAGATGCTGATGGCCCAGG; SEQ ID NO: 22) covers the initiation codon ATG and contains an Xho1 site and the reverse primer CAMEL-KPN (AAGGTACCTTGAACCGCCCCTGGTCG; SEQ ID NO: 23) contains a mutation of the stop-codon and a Kpn1 site. The vector carrying the fusion construct was transfected into COS cells by calcium phosphate precipitation, protein lysates of the cells were used for Western blotting using CAMEL antisera against the CAMEL peptides B5 and F4, and anti-EGFP antibodies to detect the fusion protein according to standard protocols.

Example 1

cDNA clone 4H8 (CAMEL) encodes the target for melanoma-specific CTL1/29

The antigenic epitope of melanoma-specific CTL 1/29 was identified by the
5 expression of cDNA library 518/IL2.14 and the restriction element HLA-A*0201 in COS-7 cells, followed by CTL screening in a TNF- α release assay. A positive pool of cDNAs was subcloned and clone 4H8, called CAMEL (SEQ ID NO: 1), was found to stimulate TNF- α release by the CTL to a similar extent as the original 518/IL2.14 cell line (Fig. 1). COS-7 cells or
10 COS-7 cells transfected with HLA-A*0201 or the 4H8 cDNA only were not recognized. The isolated 4H8 cDNA clone has a 679 bp insert, which shows strong homology with NY-ESO-1 (SEQ ID NO: 7), a tumor antigen originally identified as a target for humoral immune responses by serum screening methods (SEREX) (Chen et al., 1997). Colony hybridization of
15 the cDNA library, using clone 4H8 as a probe resulted in the detection of 2 types of full length clones which were called LAGE-1^S (SEQ ID NO: 3) and LAGE-1^L (SEQ ID NO: 5) (Fig. 2a). LAGE-1^L contains a 229 bp insertion at position 457, which has the consensus sequences for an intron, starting with a 5' GT and ending 3' AG. This indicates alternative splicing of
20 LAGE-1 mRNA. However, cDNA clone 4H8 lacks the first 84 bp of the LAGE-1 cDNA sequence.

Example 2

The peptide epitope of CTL 1/29 is coded in an alternative reading frame of LAGE-1 or NY-ESO-1

25 To identify which peptide was recognized by CTL 1/29, deletion constructs of cDNA 4H8 were transfected in HLA-A*0201⁺ COS-7 cells and tested in a TNF- α release assay. CTL reactivity was measured with all constructs (Fig. 1b), indicating that the epitope was coded within the first 330 bp of

clone 4H8. An HLA-A*0201 binding motif search was performed on the predicted protein sequence of that region (Drijfhout et al., 1995; D'Amato et al., 1995), presuming that the ATG at position 10 in 4H8 functions as the translation initiation site. Predicted strong binding peptides at regions 1-11,
 5 2-11, 1-9, 10-18, 11-19, 16-25, 17-25, 49-57, 55-63 and 70-78 of the CAMEL protein sequence (Fig. 2b) were added to HLA-A*0201⁺ BLM melanoma cells, and tested for CTL reactivity in a TNF- α release assay (Fig. 3b).

At a peptide concentration of 10 μ g/ml only the N-terminal 11- and 10-mer peptides (M)LMAQEALAF (SEQ ID NO: 11 and NO: 12) induced
 10 preponderant recognition by CTL 1/29 (Fig. 3a), indicating that the epitope recognized by the CTL is located in the first 11 amino acids of the CAMEL-encoded protein. Closer inspection of peptides derived of this N-terminal 11-mer in a peptide concentration dependent TNF- α release assay (Fig. 3b)
 15 revealed that the methionine at position 1 as well as the leucine at position 11 are of crucial importance for reconstituting CTL reactivity. Deletion of either of these amino acids leads to an at least 5 decades higher peptide concentration required for comparable TNF- α release. The only other peptide showing weak activity is the 3-11 MAQEALAF. In contrast, the
 20 MLMAQEALA has no activity at all (Fig. 3b), suggesting that the C-terminal amino acids FL do significantly contribute to the recognition by the CTL.

Example 3

Comparison of CAMEL, LAGE-1^{SL}, NY-ESO-1

As already mentioned, cDNA clone 4H8 lacks the first 84 bp of the LAGE-1^S
 25 sequence, which means that it is devoid of the initiation codon at position 54 of that sequence (Fig. 2a). The first possible translation initiation site in 4H8 corresponds with the ATG at position 94 of LAGE-1^S, which is however, not in frame with the first ATG at position 54. Therefore, the protein translated from the 4H8 cDNA clone is different from the putative

LAGE-1 protein, since translation takes place in another reading frame (Fig. 2a and b). 4H8 encodes a protein of 109 amino acids (SEQ ID NO: 2) with a predicted molecular weight of 11.7 kD. The LAGE-1^S protein translated from the first ATG will be a 180 aa protein of 18.2 kD (SEQ ID NO: 4), while
 5 the unspliced variant, LAGE-1^L, encodes a 210 aa protein of 21.1 kD (SEQ ID NO: 6). NY-ESO-1 protein (SEQ ID NO: 8) is probably of the same size as LAGE-1^S, but differs at 26 amino acids. However, if translation of LAGE-1^{SL} starts at the second ATG, proteins will be translated in another reading frame and are in that case identical to the protein translated from cDNA
 10 4H8. Alternative translation of NY-ESO-1 (SEQ ID NO: 9 and NO: 10) results in a shorter variant of this protein (58 amino acids), because of an earlier stop codon (Fig. 2b), which differs from the CAMEL protein sequence only in its last 5 amino acids (Fig. 2b).

It was examined whether cells transfected with the complete LAGE-1 (or
 15 NY-ESO-1) cDNA clones are able to stimulate CTL 1/29. Remarkably, COS/HLA-A*0201 cells transfected with LAGE-1^S, the alternatively spliced LAGE-1^L (as well as with the NY-ESO-1) cDNA are able to stimulate CTL 1/29 (Fig. 4). This indicates that, at least in COS-7 cells, protein translation also starts from the second start codon at nucleotide 94 in
 20 LAGE-1^S, notwithstanding the presence of the first ATG at position 54. Also in this case, this results in the "alternative reading frame" peptide, MLMAQEALAF_L, recognized by CTL 1/29.

Example 4

Expression of CAMEL in E. Coli

25 To investigate whether CAMEL is indeed translated from the ORF-1 of the CAMEL (4H8) cDNA, the CAMEL cDNA (SEQ ID No: 1) was cloned in a bacterial expression vector (pET19b) (Studier et al., 1990). This vector contains a 6xHis-tag coding sequence, allowing detection of the His-tagged protein with an anti-His antibody. The pET19b-CAMEL construct was

transformed into E.coli and the bacteria were treated with IPTG to induce expression of the His-tagged CAMEL protein. Extracts were analyzed by Western blotting using the Penta-His antibody. Western blotting of a lysate shows a 15.5 kD protein, only slightly higher than the expected 14.5 kD of the His-tagged CAMEL protein after staining with a anti-His antibody (Fig. 5).

The CAMEL cDNA (SEQ ID No: 1) was cloned in pET19b and expressed in E.Coli. Lanes 1 and 2 represent the samples taken at 0h, lanes 3 and 4 at 4h after induction with IPTG. Because CAMEL might be an unstable protein, induction of protein expression was performed in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of PMSF (a protease inhibitor). At the left the positions of the molecular weight marker proteins are indicated.

Example 5

15 Expression of LAGE-1 and NY-ESO-1 in healthy human tissues and melanoma cell lines

Hybridisation of Multiple Tissue Northern blots containing RNA of healthy human tissues with the LAGE-1^S cDNA showed high expression in testis and placenta and low, (but clear) expression in heart, skeletal muscle and pancreas (Fig. 6a). The positive signals exist of two bands, probably reflecting LAGE-1^S/NY-ESO-1 (750 bp) and LAGE-1^L (1000 bp).

Several melanoma cell lines were tested for expression of LAGE-1 and NY-ESO-1 by(Northern Blot analysis and) RT-PCR (Fig. 6b). Because of the strong homology between both genes, it is not possible to discriminate between LAGE-1 and NY-ESO-1 on Northern Blot. Therefore RT-PCR was performed with specific primers. In most cell lines a correlated expression of LAGE-1 and NY-ESO-1 was found; only cell line FM3.29 had expression of LAGE-1, but was negative for NY-ESO-1. Other cell lines expressed

either both or none of the two genes (Fig. 6b). There was a good correlation between the level of expression and the recognition by CTL 1/29 (Fig. 6b).

Example 6

5 Determination of LAGE1/CAMEL expression of in human tumors by RT-PCR

In order to evaluate the percentage of LAGE1/CAMEL positive human tumors, individual tumor tissues from breast or lung cancer patients were subjected to RT-PCR, as described in the Method section f), using the LAGE1-specific primers F3 (SEQ ID NO: 17) and ESO-1B (SEQ ID
10 NO: 18); reactions were performed in a Perkin Elmer 9600 thermocycler with 35 (instead of 30) cycles.

As shown in Table 1, approximately 50% (3/6) of the tested breast cancer specimens and 80% (10/12) of the lung cancer specimens were shown to be positive for LAGE1/CAMEL mRNA .

WO 00/23584

PCT/EP99/07832

24

Table 1:

<i>Tumor type</i>	<i>#</i>	<i>LAGE1/CAMEL mRNA</i>
breast (ILC)	71526 / 85	-
breast (ILC)	44231 / 95	-
breast (ILC)	73507 / 95	+
breast (IDC)	19837 / 95	++
breast (IDC)	4385 / 95	+
breast (IDC)	48897 / 95	-
lung (AC)	15827 / 97	+
lung (AC)	53934 / 97	++
lung (AC)	67086 / 93	++
lung (AC)	62357 / 96	+
lung (AC)	T63244 / 93	-
lung (AC)	T64360 / 93	+
lung (SCC)	92710 / 96	++
lung (SCC)	53005	++
lung (SCC)	28649 / 97	-
lung (SCC)	16251 / 97	+
lung (SCC)	5063 / 93	++
lung (SCC)	7580 / 97	+
testis (positive control)	(GibcoBRL)	+

Example 7

Immunohistochemical analysis of CAMEL expression in human tumors

As described in Examples 5 and 6, LAGE-1 mRNA was detected in a panel
5 of tumor cell lines and tumor tissues and in a restricted number of healthy
tissues by means of RT-PCR and Northern blot experiments. However, it
remained to be determined whether in cells expressing the LAGE-1 mRNA
also the alternatively translated CAMEL is produced.

Frozen sections from a panel of different human tumors were analyzed by
10 immunohistochemistry using a CAMEL-specific rabbit antiserum B5 which
was affinity-purified against the B5 peptide. (For the preparation of the
antisera, see h) in the Methods section. The B5 antiserum was used
because B5 is CAMEL-specific, while the F4 antiserum may also recognize
an epitope present on a protein expressed from the ORF-1 of NY-ESO-1).
15 Specificity of the purified serum was demonstrated by peptide ELISA and
Western blotting against COS cells transfected with a CAMEL-GFP fusion
protein. Immunohistochemistry was performed using a 3-step avidin-biotin-
peroxidase staining procedure. The results are summarized in Table 2,
examples are shown in Figure 7.

Table 2:

Tumor type (n=number of cases)	Percentage of positive tumor cells				
	negative	< 10%	10-40%	41-70%	>70%
Breast AC (n=11)	2	4	1	2	2
Colon AC (n=8)	1	0	1	1	5
Lung AC (n=10)	2	2	2	3	1
Lung SCC (n=9)	4	3	1	1	0
Pancreas AC (n=10)	1	2	0	2	5

AC: adenocarcinoma

SCC: squamous cell carcinoma

5

A total of 48 different specimen was investigated. In the majority of cases (38/48) CAMEL expression was detected. About half of the positive cases showed expression of CAMEL in 40% or more of the tumor cells, in some of the cases close to 100% of the tumor cells showed CAMEL-specific staining (an example is shown Figure 7, Colon AC). In the majority of tumor specimens expression was heterogeneous ranging from less than 10% of positive tumor cells to more than 70% of positive tumor cells (Table 2; examples are shown in Figure 7, arrows indicate positive tumor cell staining).

15 Example 8

Identification of HLA-A2 binding peptides within the CAMEL ORF

In order to identify further HLA-A2 epitopes besides the CTL-epitope (M)LMAQEALAF (SEQ ID NO:11 and 12), CAMEL (SEQ ID NO:2) was examined according to the algorithms and motifs published by Parker et al.;

1994, and Rammensee et al., 1995. The result of this analysis indicated that several further peptides within the CAMEL protein have the potential to bind to HLA-A2, and three of these candidates -CAMEL10: FLMAQGAML (SEQ ID NO: 24), CAMEL16: AMLAAQERRV (SEQ ID NO: 25) and
5 CAMEL17: MLAAQERRV (SEQ ID NO:26) – were synthesized.

These synthetic peptides were evaluated for their ability to increase surface HLA-A2 expression on the transport defective cell line 174CEM.T2 (Nijman et al., 1993). Briefly, 5×10^5 cells/0.2 ml/well were seeded in 96-well V-bottom plates and incubated for 16 hours with increasing amounts
10 (0-320 µg/ml) of peptide at 37°C in a humidified atmosphere. HLA-A2 surface expression was measured by FACS analysis (Becton Dickinson) using purified BB7.2 as primary antibody and a goat-anti-mouse IgG RPE conjugate(DAKO) as detection antibody.

As positive controls the known HLA-A2 restricted CTL-epitopes from
15 CAMEL (MLMAQEALAF, SEQ ID NO:11) or tyrosinase (Wölfel et al., 1994; YMNGTMSQV, SEQ ID NO:27) were applied. Negative controls included an HLA-A1 binding (and therefore irrelevant) peptide from MAGE-3 (Gaugler et al. , 1994; EVDPIGHLY, SEQ ID NO:28) or no peptide at all.

20 The results from these experiments suggest that the nonamer CAMEL10 binds with similar affinity to HLA-A2 as compared to the positive controls used in the assay. The two other peptides (CAMEL16 and CAMEL17) showed only low affinity in this assay. Therefore in particular CAMEL10 represents a potential new HLA-A2 restricted CTL-epitope derived from
25 CAMEL protein (FIG. 8).

The testing of the immunogenicity of CAMEL10 and if it represents a naturally processed and presented ligand can be done as described in WO 97/30721 and Schweighoffer, 1997.

Bibliography

- Bakker, A.B.H., van der Burg, S.H., Huijbens, R.J.F., Drijfhout, J.-W.,
 5 Melief, J.M., Adema, G.J., and Figdor, C.G. (1997). Analogues of CTL
 epitopes with improved MHC Class-I Binding capacity elicit anti-melanome
 CTL recognizing the wild-type epitope. *Int. J. Cancer* 70, 302-309.
- Blake, J., Johnston, J.V., Hellström, K.E., Marquardt, H., and, Chen, L.
 10 (1996). Use of Combinatorial Peptide Libraries to Construct
 Functional Mimics of Tumor Epitopes Recognized by MHC Class I-
 Restricted Cytolytic T Lymphocytes. *J. Exp. Med.* 184, 121-130.
- Chen, Y.T., Scanlan, M.J., Sahin, U., Tureci, O., Gure, A.O., Tsang, S.,
 15 Williamson, B., Stockert, E., Pfreundschuh, M., and Old, L.J. (1997). A
 testicular antigen aberrantly expressed in human cancers detected by
 autologous antibody screening. *Proc. Natl. Acad. Sci. U. S. A.* 94,
 1914-1918.
- 20 Coulie P.G. (1997) Human tumour antigens recognized by T cells: new
 perspectives for anti cancer vaccines. *Mol. Med. Today* 3, 261-268
- D'Amaro, J., Houbiers, J.G., Drijfhout, J.W., Brandt, R.M., Schipper, R.,
 Bavinck, J.N., Melief, C.J., and Kast, W.M. (1995). A computer program for
 25 predicting possible cytotoxic T lymphocyte epitopes based on HLA class I
 peptide-binding motifs. *Hum. Immunol.* 43, 13-18.
- Dranoff, G., Jaffee, E., Lazenby, A., Golumbek, P., Levitsky, H., Brose, K.,
 Jackson, V., Hamada, H., Pardoll, D., and Mulligan, R. (1993). Vaccination
 30 with irradiated tumor cells engineered to secrete murine
 granulocyte-macrophage colony-stimulating factor stimulates potent,

specific, and long-lasting anti-tumor immunity. *Proc. Natl. Acad. Sci. USA* **90**, 3539-3543.

Drijfhout, J.W., Brandt, R.M., D'Amaro, J., Kast, W.M., and Melief, C.J.
5 (1995). Detailed motifs for peptide binding to HLA-A*0201 derived from large random sets of peptides using a cellular binding assay. *Hum. Immunol.* **43**, 1-12.

Falk, K., Rötzschke, O., Stevanović, S., Jung, G., and Rammensee, H-G
10 (1991). Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature* **351**, 290-296.

Fearon, E.R., Pardoll, D.M., Itaya, T., Golumbek, P., Levitsky, H.I., Simons, J.W., Karasuyama, H., Vogelstein, B., and Frost, P. (1990). Interleukine-2
15 production by tumor cells bypasses T helper function in the generation of an antitumor response. *Cell* **60**, 397-403.

Gansbacher, B., Zier, K., Daniels, B., Cronin, K., Bannerji, R., and Gilboa, E. (1990). Interleukin 2 gene transfer into tumor cells abrogates
20 tumorigenicity and induces protective immunity. *J. Exp. Med.* **172**, 1217-1224.

Gaugler B, van den Eynde B, van der Bruggen P, Romero P, Gaforio JJ, De Plaen E, Lethe B, Brasseur F, Boon T (1994) *J. Exp. Med.* **179**:921-30
25

Jäger, E., Chen, Y.T., Drijfhout, J.W., Karbach, J., Ringhoffer, M., Jäger, D., Arand, M., Wada, H., Noguchi, Y., Stockert, E., Old, L.J., and Knuth, A. (1998). Simultaneous humoral and cellular immune response against cancer-testis antigen NY-ESO-1: definition of human histocompatibility
30 leukocyte antigen (HLA)-A2-binding peptide epitopes. *J. Exp. Med.* **187**, 265-270.

- Kawakami, Y., Eliyahu, S., Jennings, C., Sakaguchi, K., King, X., Southwood, S., Robbins, P.F., Sette, A., Appella, E., and Rosenberg, S.A. (1995). Recognition of multiple epitopes in the human melanoma antigen gp100 by tumor-infiltrating T lymphocytes associated with *in vivo* tumor regression. J. Immunol. 154, 3961-3968.
- 5 Lethe, B., Lucas, S., Michaux, L., Desmet, C., Godelaine, D., Serrano, A., Deplaen, E. and Boon, T. (1998). LAGE-1, a new gene with tumor specificity. Int. J. Cancer 76 (6):903-908.
- 10 Lin, A.Y., Devaux, B., Green, A., Sagerström, C., Elliott, J.F., and Davis, M.M. (1990). Expression of T Cell Antigen Receptor Heterodimers in a Lipid-Linked Form. Science 249, 677-679.
- 15 Mayordomo, J.I., Zorina, T., Storkus, W.J., Zitvogel, L., Celluzzi, C., Falo, L.D., Melief, C.J., Ildstad, S.T., Kast, W.M., DeLeo, A.B., and Lotze, M.T. (1995). Bone marrow-derived dendritic cells pulsed with synthetic tumour peptides elicit protective and therapeutic antitumour immunity. Nature Medicine 1, 1297-1302.
- 20 Nijman HW, Houbiers JG, Vierboom MP, van der Burg SH, Drijfhout JW, D'Amato J, Kenemans P, Melief CJ, Kast WM (1993) Eur. J. Immunol. 6: 1215-9
- 25 Osanto, S., Brouwenstyn, N., Vaessen, N., Figdor, C.G., Melief, C.J., and Schrier, P.I. (1993). Immunization with interleukin-2 transfected melanoma cells. A phase I-II study in patients with metastatic melanoma. Hum. Gene Ther. 4, 323-330.
- 30 Parker KC, Bednarek MA, Coligan JE (1994), J. Immunol. 152:163

- Parkhurst, M.R., Salgaller, M.L., Southwood, S., Robbins, P.F., Sette, A., Rosenberg, S.A., and Kawakami, Y. (1996). Improved induction of melanoma-reactive CTL with peptides from the melanoma antigen gp100 modified at HLA-A*0201-binding residues. *J. Immunol.* **157**, 2539-2548.
- 5 Rammensee, H.G. and Friede, T. (1995). MHC ligands and peptide motifs: first listing. *Immunogenetics* **41**, 179-228.
- Ruppert, J., Sidney, J., Celis, E., Kubo, R.T., Grey, H.M., and Sette, A. (1993). Prominent role of secondary anchor residues in peptide binding to HLA-A2.1 molecules. *Cell* **74**, 929-937.
- 10 Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). *Molecular Cloning, a Laboratory Manual* (New York: Cold Spring Harbor Laboratory Press).
- 15 Schweighoffer, T. (1997) Molecular cancer vaccines: tumor therapy using antigen-specific immunizations. *Path. Onc. Res.* **3**, 164-176
- Sette, A., Vitiello, A., Rehman, B., Fowler, P., Nayersina, R., Kast, W.M., Melief, C.J.M., Oseroff, C., Yuan, L., Ruppert, J., Sidney, J., del Guercio, M.-F., Southwood, S., Kubo, R.T., Chesnut, R.W., Grey, H.M., and Chisari, F.V. (1994). The relationship between class I binding affinity and immunogenicity of potential cytotoxic T Cell epitopes. *J. Immunol.* **153**, 5586-5592.
- 20 Stauss, H.J., Davies, H., Sadovnikova, E., Chain, B., Horowitz., and Sinclair, C. (1992). Induction of cytotoxic T lymphocytes with peptides *in vitro*: Identification of candidate T-cell epitopes in human papilloma virus. *Proc. Natl. Acad. Sci. USA.* **89**, 7871-7875.
- 25

Studier, F.W., Rosenberg, A.H., Dunn, J.J. and Dubendorf, J.W. (1990).
Use of T7 RNA polymerase to direct expression of cloned genes. *Methods
in Enzymology* 185, 60-89

- 5 Tepper, R.I., Pattengale, P.K., and Leder, P. (1989). Murine interleukine-4
displays potent anti-tumour activity *in vivo*. *Cell* 57, 503-512.

- Toes, R.E., Hoebe, R.C., Van der Voort, E., Rensing, M.E., Van-der-Eb,
A.J. Melief, C.J.M., and Offringa, R. 1997 Protective anti-tumor immunity
10 induced by vaccination with recombinant adenoviruses encoding multiple
tumor-associated cytotoxic T lymphocyte epitopes in a string-of-beads
fashion. *Proc. Natl. Acad. Sci. U.S.A.* 94 (26):14660-14665

- Tuting, T., DeLeo, A.B., Lotze, M.T., and Storkus, W.J. Genetically modified
15 bone marrow-derived dendritic cells expressing tumor-associated viral or
"self" antigens induce antitumor immunity *in vivo*. *Eur. J. Immunol.* 27,
2702-2707, 1997.

- van der Burg, S.H., et al., (1996), Immunogenicity of peptides bound to
20 MHC class I molecules depends on the MHC-peptide complex stability.
J. Immunol. 156, 3308-3314

- Van den Eynde, B. and Brichard, V.G. (1995). New tumor antigens
recognized by T cells. *Current Opinion in Immunology* 7, 674-681.

- 25 van Elsas, A., van der Minne, C.E., Borghi, M., van der Spek, C.W.,
Braakman, E., Osanto, S., and Schrier, P.I. (1996). CTL Recognition of an
IL-2 Producing Melanoma Vaccine. In: *Immunology of human melanoma.*
Tumor-host interaction and immunotherapy, edited by M. Maio, Amsterdam:
30 IOS, 1996, p. 165-173.

- van Elsas, A., Aamoudse, C., van der Minne, C.E., van der Spek, C.W.,
Brouwenstijn, N., Osanto, S., and Schrier, P.I. (1997). Transfection of IL-2
augments CTL response to human melanoma cells *in vitro*: immunological
characterization of a melanoma vaccine. *Journal of Immunotherapy* 20,
5 343-353.
- Versteeg, R., Noordermeer, I.A., Krüse-Wolters, K.M., Ruiter, D.J., and
Schrier, P.I. (1988). c-Myc downregulates class I HLA expression in human
melanomas. *EMBO J.* 7, 1023-1029.
- 10 Wang, R.F., Johnston, S.L., Zeng, G., Topalian, S.L., Schwartzentruber, D.J.,
and Rosenberg, S.A. (1998). A breast and melanoma-shared tumor antigen:
T cell responses to antigenic peptides translated from different open
reading frames. *J. Immunol.* 161, 3596-3606.
- 15 Wölfel T, van Pel A, Brichard V, Schneider J, Seliger B, Meyer zum
Büschenfelde KH, Boon T (1994) *Eur. J. Immunol.* 24:759-64
- 20 Wu, T.C., Guarnieri, F.G., Staveley-O'Carroll, K.F., Viscidi, R.P., Levitsky,
H.I., Hedrick, L., Cho, K.R., August, J.T., and Pardoll, D.M. (1995).
Engineering an intracellular pathway for major histocompatibility complex
class II presentation of antigens. *Proc. Natl. Acad. Sci. U.S.A.* 92(25):
11671-11675.
- 25 Zitvogel, L., Mayordomo, J.I., Tjandrawan, T., DeLeo, A.B., Clarke, M.R.,
Lotze, M.T., and Storkus, W.J. (1996). Therapy of murine tumors with tumor
peptide-pulsed dendritic cells: dependence on T cells, B7 costimulation,
and T helper cell 1-associated cytokines. *J. Exp. Med.* 183, 87-97.

WO 00/23584

PCT/EP99/07832

1/18

SEQUENCE LISTING

5

(1) GENERAL INFORMATION:

10

(i) APPLICANT:

- (A) NAME: Boehringer Ingelheim International GmbH
- (B) STREET: Binger Strasse 173
- (C) CITY: Ingelheim am Rhein
- (E) COUNTRY: Germany
- (F) POSTAL CODE (ZIP): 55216
- (G) TELEPHONE: 06132/772282
- (H) TELEFAX: 06132/774377

15

20

(ii) TITLE OF INVENTION: Tumor-associated Antigen

(iii) NUMBER OF SEQUENCES: 28

25

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

30

35

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 679 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: cDNA to mRNA

45

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

50

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: homo sapiens
- (F) TISSUE TYPE: Melanoma

55

(ix) FEATURE:

- (A) NAME/KEY: 3'UTR

WO 00/23584

PCT/EP99/07832

2/18

(B) LOCATION:340..679

(ix) FEATURE:

5

(A) NAME/KEY: 5'UTR

(B) LOCATION:1..9

(ix) FEATURE:

10

(A) NAME/KEY: CDS

(B) LOCATION:10..339

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

15	CGACGGGCG ATG CTG ATG GGC CAG GAG GGC CTG GCA TTC CTG ATG GGC	48
	Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu Met Ala	
	1 5 10	
20	CAG GGG GCA ATG CTG GCG GGC CAG GAG AGG CCG GTG CCA CCG GCG GCA	96
	Gln Gly Ala Met Leu Ala Ala Gln Glu Arg Arg Val Pro Arg Ala Ala	
	15 20 25	
25	GAG GTC CCG GGG GCG CAG GGG CAG CAA GGG CCT CCG GGC CGA GAG GAG	144
	Glu Val Pro Gly Ala Gln Gly Gln Gln Gly Pro Arg Gly Arg Glu Glu	
	30 35 40 45	
30	GCG CCG CCG GGG GTC CCG ATG GCG GTG CCG CTT CTG CCG AGG ATG GAA	192
	Ala Pro Arg Gly Val Arg Met Ala Val Pro Leu Leu Arg Arg Met Glu	
	50 55 60	
35	GGT GGC CCT GCG GGG CCA GGA GGC CCG ACA GGC GGC TGC TTC AGT TGC	240
	Gly Ala Pro Ala Gly Pro Gly Gly Arg Thr Ala Ala Cys Phe Ser Cys	
	65 70 75	
40	ACA TCA CGA TGC CTT TCT CGT CCG CCA TGG AAG CCG AGC TGG TCC GCA	288
	Thr Ser Arg Cys Leu Ser Arg Arg Pro Trp Lys Arg Ser Trp Ser Ala	
	80 85 90	
45	GGA TCC TGT CCG GGG ATG CCG CAC CTC TCC CCG GAC CAG GGG CCG TTC	336
	Gly Ser Cys Pro Gly Met Pro His Leu Ser Pro Asp Gln Gly Arg Phe	
	95 100 105	
50	TGA AGGACTTCAC CGTGTGCGGC AACCTACTGT TTATCGACT GACTGCTGCA	389
	*	
55	110	
	GACCAACGCG AACTCAGCT CTCCATCAGC TCTGTCTCC AGCAGCTTC CCGTGTGATG	449
	TGATCAGC AGTGTCTTCT GCGGTGTTT TTGGCTCAGG CTCCCTCAGG GCAGAGGCGC	509
	TAAGCCAGC CTGGGCGGCC TTCTAGGTC ATGCTCTCTC CCTAGGGAA TGGTCCAGC	569
	ACGAGTGGC AGTCAATGT GGGGGCTGA TTGTTGTGG CTGGAGGAG AGGCTTACA	629
	TGTTTGTTC TGAGAAAAT AAGCTGAGC TACGAAAAA AAAAAAAAAA	679

WO 00/23584

3/18

PCT/EP99/07832

(2) INFORMATION FOR SEQ ID NO: 2:

- 5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 109 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

- 10 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

15 Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu Met Ala Gln Gly Ala
 1 5 10 15
 Met Leu Ala Ala Gln Glu Arg Arg Val Pro Arg Ala Ala Glu Val Pro
 20 25 30
 20 Gly Ala Gln Gly Gln Gln Gly Pro Arg Gly Arg Glu Glu Ala Pro Arg
 35 40 45
 Gly Val Arg Met Ala Val Pro Leu Leu Arg Arg Met Glu Gly Ala Pro
 50 55 60
 25 Ala Gly Pro Gly Gly Arg Thr Ala Ala Cys Phe Ser Cys Thr Ser Arg
 65 70 75 80
 30 Cys Leu Ser Arg Arg Pro Trp Lys Arg Ser Trp Ser Ala Gly Ser Cys
 85 90 95
 Pro Gly Met Pro His Leu Ser Pro Asp Gln Gly Arg Phe *
 100 105 110

35

(2) INFORMATION FOR SEQ ID NO: 3:

- 40 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 767 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- 45 (ii) MOLECULE TYPE: cDNA to mRNA

- (iii) HYPOTHETICAL: NO

- 50 (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: homo sapiens
 (F) TISSUE TYPE: Melanoma

- 55 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 54..596

WO 00/23584

4/18

PCT/EP99/07832

(ix) FEATURE:

(A) NAME/KEY: 3'UTR

(B) LOCATION:597..767

5

(ix) FEATURE:

(A) NAME/KEY: 5'UTR

(B) LOCATION:1..53

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

	ATCTCTGTGG GCGCTGACCT TCTCTCTGAG AGCGGGGCGAG AGGCTCAGGA GGC ATG	56
15		Met 1
	CAG GGC GAA GGC CAG GGC ACA GGG GGT TGG ACG GGC GAT GCT GAT GGC	104
	Gln Ala Glu Gly Gln Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp Gly	
20		5 10 15
	CCA GGA GGC CCT GGC ATT OCT GAT GGC CCA GGG GGC AAT GCT GGC GGC	152
	Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly Gly	
		20 25 30
25	CCA GGA GAG GCG GGT GGC ACG GGC GGC AGA GGT CCC CGG GGC GCA GGG	200
	Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala Gly	
		35 40 45
30	GCA GCA AGG GGC TGG GGG CCG AGA GGA GGC GGC CCG CCG GGT CCG CAT	248
	Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro His	
		50 55 60 65
	GGC GGT GGC GCT TCT GCG CAG GAT GGA AGG TGC CCC TGC GGG GGC AGG	296
35	Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala Arg	
		70 75 80
	AGG CCG GAC AGC CCG CTG CTT CAG TTG CAC ATC ACG ATG OCT TTC TCG	344
	Arg Pro Asp Ser Arg Leu Leu Gln Leu His Ile Thr Met Pro Phe Ser	
		85 90 95
40	TGG CCC ATG GAA GCG GAG CTG GTC CCG AGG ATC CTG TCC CCG GAT GGC	392
	Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp Ala	
		100 105 110
45	GCA OCT CTC CCC CGA CCA GGG GCG GTT CTG AAG GAC TTC ACC GTG TCC	440
	Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val Ser	
		115 120 125
50	GGC AAC CTA CTG TTT ATC CGA CTG ACT GCT GCA GAC CAC CCG CAA CTG	488
	Gly Asn Leu Leu Phe Ile Arg Leu Thr Ala Ala Asp His Arg Gln Leu	
		130 135 140 145
	CAG CTC TCC ATC AGC TCC TGT CTC CAG CAG CTT TCC CTG TTG ATG TGG	536
55	Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met Trp	
		150 155 160

WO 00/23584

PCT/EP99/07832

5/18

	ATC ACG CAG TGC TTT CTG CCC GTG TTT TTG GCT CAG GCT CCC TCA GGG	584
	Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Ala Pro Ser Gly	
	165 170 175	
5	CAG AGG CGC TAA GGCAGGCTG GGGGGGCTC CTAGGTCATG CCTCTGCCC	636
	Gln Arg Arg *	
	180	
10	TAGGGAATGG TCCAGCAAG AGTGGCCAGT TCATTGTGGG GGCCGATTG TTGTGCGCTG	696
	GAGGAGGACG GCTTACATGT TTGTTCTGT AGAAAATAAA GCTGAGCTAC GAAAAAAAAA	756
	AAAAAAAAA A	767
15		
	(2) INFORMATION FOR SEQ ID NO: 4:	
20	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 180 amino acids	
	(B) TYPE: amino acid	
	(D) TOPOLOGY: linear	
25	(ii) MOLECULE TYPE: protein	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:	
30	Met Gln Ala Glu Gly Gln Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp	
	1 5 10 15	
	Gly Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly	
	20 25 30	
35	Gly Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala	
	35 40 45	
	Gly Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro	
	50 55 60	
40	His Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala	
	65 70 75 80	
	Arg Arg Pro Asp Ser Arg Leu Leu Gln Leu His Ile Thr Met Pro Phe	
45	85 90 95	
	Ser Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp	
	100 105 110	
50	Ala Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val	
	115 120 125	
	Ser Gly Asn Leu Leu Phe Ile Arg Leu Thr Ala Ala Asp His Arg Gln	
	130 135 140	
55	Leu Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met	
	145 150 155 160	

WO 00/23584

PCT/EP99/07832

6/18

Trp Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Ala Pro Ser
 165 170 175

5 Gly Gln Arg Arg *
 180

(2) INFORMATION FOR SEQ ID NO: 5:

10

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 993 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 15 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

20

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

25

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: homo sapiens
 (F) TISSUE TYPE: Melanoma

30

(ix) FEATURE:
 (A) NAME/KEY: 5'UTR
 (B) LOCATION:1..55

35

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION:56..688

40

(ix) FEATURE:
 (A) NAME/KEY: 3'UTR
 (B) LOCATION:689..993

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

45

GCATCTCTGT GGGGCTGAC CTCTCTCTG AGAGCGGGC AGAGGCTGG GAGCC ATG 58
 Met
 1

50

CAG GGC GAA GGC CAG GGC ACA GGG GGT TGG ACG GGC GAT GCT GAT GGC 106
 Gln Ala Glu Gly Gln Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp Gly
 5 10 15

55

CCA GGA GGC OCT GGC ATT OCT GAT GGC CCA GGG GGC AAT GCT GGC GGC 154
 Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly Gly
 20 25 30

CCA GGA GAG GCG GGT GGC ACG GGC GGC AGA GGT CCC CGG GGC GCA GGC 202
 Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala Gly
 35 40 45

GCA GCA AGG GGC TGG GGG CCG AGA GGA GGC GGC CCG CGG GGT CCG CAT 250

WO 00/23584

PCT/EP99/07832

7/18

	Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro His	
	50 55 60 65	
5	GGC GGT GGC GCT TCT GCG CAG GAT GGA AGG TGC CCC TGC GGG GGC AGG Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala Arg	298
	70 75 80	
10	AGG CCG GAC AGC GCG CTG CTT CAG TTG CAC ATC ACG ATG OCT TTC TCG Arg Pro Asp Ser Arg Leu Leu Gln Leu His Ile Thr Met Pro Phe Ser	346
	85 90 95	
15	TOG CCC ATG GAA GCG GAG CTG GTC GCG AGG ATC CTG TOC CCG GAT GGC Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp Ala	394
	100 105 110	
20	GCA CCT CTC CCC CGA CCA GCG GCG GTT CTG AAG GAC TTC ACC GTG TOC Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val Ser	442
	115 120 125	
25	GGC AAC CTA CTG TTT ATG TCA GTT CCG GAC CAG GAC AGG GAA GGC GCT Gly Asn Leu Leu Phe Met Ser Val Arg Asp Gln Asp Arg Glu Gly Ala	490
	130 135 140 145	
30	GGG CCG ATG AGG GTG GTG GGT TGG GCG CTG GGA TOC GGC TOC CCG GAG Gly Arg Met Arg Val Val Gly Trp Gly Leu Gly Ser Ala Ser Pro Glu	538*
	150 155 160	
35	GGG CAG AAA GCT AGA GAT CTC AGA ACA CCC AAA CAC AAG GTC TCA GAA Gly Gln Lys Ala Arg Asp Leu Arg Thr Pro Lys His Lys Val Ser Glu	586
	165 170 175	
40	CAG AGA CCT GGT ACA CCA GCG CCG CCG CCA CCC GAG GGA GGC CAG GGA Gln Arg Pro Gly Thr Pro Gly Pro Pro Pro Glu Gly Ala Gln Gly	634
	180 185 190	
45	GAT GGG TGC AGA GGT GTC GGC TTT AAT GTG ATG TTC TCT GGC CCT CAC Asp Gly Cys Arg Gly Val Ala Phe Asn Val Met Phe Ser Ala Pro His	682
	195 200 205	
50	ATT TAG CCGACTGACT GCTGCAGAC ACGGCCAACT GCAGCTCTCC ATCAGCTCT Ile *	738
	210	
55	GCTCCAGCA GCTTTCCCTG TTGATGTGGA TCAGGCAGTG CTTTCTGCCC GTGTTTGTG	798
	CTCAGGCTCC CTCAGGCGAG AGGCGCTAAG CCCAGCTCTG CGCCCTCTCC TAGGTCATGC	858
	CTCCCTCCCT AGGGAATGTT CCAGCAAGA GTGGCCAGTT CATTTGTTGGG GCTGATGTG	918
	TTGTGCTCTG AGGAGGAAGG CTACATGTT TGTTCCTGTA GAAATTAAG CTGAGCTAAG	978
	AAAAAAAAAAAA	993

(2) INFORMATION FOR SEQ ID NO: 6:

WO 00/23584

PCT/EP99/07832

8/18

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 210 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

10 Met Gln Ala Glu Gly Gln Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp
 1 5 10 15
 Gly Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly
 20 25 30
 15 Gly Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala
 35 40 45
 Gly Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro
 20 50 55 60
 His Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala
 65 70 75 80
 25 Arg Arg Pro Asp Ser Arg Leu Leu Gln Leu His Ile Thr Met Pro Phe
 85 90 95
 Ser Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp
 100 105 110
 30 Ala Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val
 115 120 125
 Ser Gly Asn Leu Leu Phe Met Ser Val Arg Asp Gln Asp Arg Glu Gly
 35 130 135 140
 Ala Gly Arg Met Arg Val Val Gly Trp Gly Leu Gly Ser Ala Ser Pro
 145 150 155 160
 40 Glu Gly Gln Lys Ala Arg Asp Leu Arg Thr Pro Lys His Lys Val Ser
 165 170 175
 Glu Gln Arg Pro Gly Thr Pro Gly Pro Pro Pro Glu Gly Ala Gln
 180 185 190
 45 Gly Asp Gly Cys Arg Gly Val Ala Phe Asn Val Met Phe Ser Ala Pro
 195 200 205
 His Ile *
 50 210

55

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

WO 00/23584

9/18

PCT/EP99/07832

(A) LENGTH: 752 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

10 (iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

15 (ix) FEATURE:
 (A) NAME/KEY: 5'UTR
 (B) LOCATION:1..53

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION:54..596

20 (ix) FEATURE:
 (A) NAME/KEY: 3'UTR
 (B) LOCATION:597..752

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

30	ATCTCTGTGG GGCCTGAOCT TCTCTCTGAG AGCGGGGCGAG AGGCTCCTGGA GGC ATG	56
	Met	
	1	
35	CAG GGC GAA GGC CGC GGC ACA GGG GGT TGG ACG GGC GAT GCT GAT GGC	104
	Gln Ala Glu Gly Arg Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp Gly	
	5 10 15	
40	CCA GGA GGC OCT GGC ATT OCT GAT GGC CCA GGG GGC AAT GCT GGC GGC	152
	Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly Gly	
	20 25 30	
45	CCA GGA GAG GCG GGT GGC ACG GGC GGC AGA GGT CCC CGC GGC GCA GGC	200
	Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala Gly	
	35 40 45	
50	GCA GCA AGG GGC TGG GGG CCG GGA GGA GGC GGC CCG CCG GGT CCG CAT	248
	Ala Ala Arg Ala Ser Gly Pro Gly Gly Gly Ala Pro Arg Gly Pro His	
	50 55 60 65	
55	GGC GGC GCG GCT TCA GGG CTG AAT GGA TGC TGC AGA TGC GGC GGC AGG	296
	Gly Gly Ala Ala Ser Gly Leu Asn Gly Cys Cys Arg Cys Gly Ala Arg	
	70 75 80	
55	GGG CCG GAG AGC CGC CTG CIT GAG TTC TAC CTC GGC ATG OCT TTC GCG	344
	Gly Pro Glu Ser Arg Leu Leu Glu Phe Tyr Leu Ala Met Pro Phe Ala	
	85 90 95	
	ACA CCC ATG GAA GCA GAG CTG GGC CGC AGG AGC CTG GGC CAG GAT GGC	392

WO 00/23584

10/18

PCT/EP99/07832

Thr Pro Met Glu Ala Glu Leu Ala Arg Arg Ser Leu Ala Gln Asp Ala
 100 105 110

5 CCA CCG CTT CCC GTG CCA GGG GTG CTT CTG AAG GAG TTC ACT GTG TOC 440
 Pro Pro Leu Pro Val Pro Gly Val Leu Leu Lys Glu Phe Thr Val Ser
 115 120 125

10 GGC AAC ATA CTG ACT ATC CCA CTG ACT GCT GCA GAC CAC CCC CAA CTG 488
 Gly Asn Ile Leu Thr Ile Arg Leu Thr Ala Ala Asp His Arg Gln Leu
 130 135 140 145

CAG CTC TOC ATC AGC TOC TGT CTC CAG CAG CTT TOC CTG TTG ATG TGG 536
 Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met Trp
 150 155 160

15 ATC AGC CAG TGC TTT CTG CCC GTG TTT TTG GCT CAG OCT CCC TCA GGG 584
 Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Pro Pro Ser Gly
 165 170 175

20 CAG AGG CCC TAA GGCAGGCTG GGGGGCCCTC CTAGGTCATG CCTCTCCCC 636
 Gln Arg Arg *
 180

25 TAGGGAATGG TOCCAGCAGG AGTGGCCAGT TCATTGTGGG GGCTGATG TTGTGGCTG 696
 GAGGAGCAGG GCTTACATGT TTGTTTCGT AGAAATATAA ACTGAGCTAC GAAAAA 752

30

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

35

- (A) LENGTH: 180 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Met Gln Ala Glu Gly Arg Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp
 1 5 10 15

45 Gly Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly
 20 25 30

Gly Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala
 35 40 45

50 Gly Ala Ala Arg Ala Ser Gly Pro Gly Gly Gly Ala Pro Arg Gly Pro
 50 55 60

55 His Gly Gly Ala Ala Ser Gly Leu Asn Gly Cys Cys Arg Cys Gly Ala
 65 70 75 80

Arg Gly Pro Glu Ser Arg Leu Leu Glu Phe Tyr Leu Ala Met Pro Phe
 85 90 95

SUBSTITUTE SHEET (RULE 26)

WO 00/23584

PCT/EP99/07832

11/18

Ala Thr Pro Met Glu Ala Glu Leu Ala Arg Arg Ser Leu Ala Gln Asp
 100 105 110

5 Ala Pro Pro Leu Pro Val Pro Gly Val Leu Leu Lys Glu Phe Thr Val
 115 120 125

Ser Gly Asn Ile Leu Thr Ile Arg Leu Thr Ala Ala Asp His Arg Gln
 130 135 140

10 Leu Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met
 145 150 155 160

15 Trp Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Pro Pro Ser
 165 170 175

Gly Gln Arg Arg *
 180

20

(2) INFORMATION FOR SEQ ID NO: 9:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 752 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

35

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: homo sapiens

40

(ix) FEATURE:

(A) NAME/KEY: 5'UTR

(B) LOCATION:1..93

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:94..270

45

(ix) FEATURE:

(A) NAME/KEY: 3'UTR

(B) LOCATION:271..752

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

55 ATCTCTGTGG GGGTGAAGT TCTCTCTGAG AGGCGGGGAG AGGCTGCGGA GGCATGCGG 60

CGGAGGGGCG GGGGACAGGG GGTTCGAGGG GCG ATG CTG ATG GGC CAG GAG GGC 114

Met Leu Met Ala Gln Glu Ala

WO 00/23584

PCT/EP99/07832

12/18

		1	5	
	CTG GCA TTC CTG ATG GGC CAG GGG GCA ATG CTG GCG GGC CAG GAG AGG			162
5	Leu Ala Phe Leu Met Ala Gln Gly Ala Met Leu Ala Ala Gln Glu Arg			
	10 15 20			
	CGG GTG CCA CCG GCG GCA GAG GTC CCC GGG GCG CAG GGG CAG CAA GGG			210
	Arg Val Pro Arg Ala Ala Glu Val Pro Gly Ala Gln Gly Gln Gln Gly			
10	25 30 35			
	CGT CCG GGC CCG GAG GAG GCG CCC CCG GGG GTC CCG ATG GCG GCG CCG			258
	Pro Arg Gly Arg Glu Glu Ala Pro Arg Gly Val Arg Met Ala Ala Arg			
	40 45 50 55			
15	CTT CAG GGC TGA ATGGATGCTG CAGATGCGGG GCGAGGGGGC CCGAGAGCGG			310
	Leu Gln Gly *			
20	CGTGGTGGAG TTCTAAGCTG CCATGCGTTT CGGACACGCC ATGGAGGCAG AGCTGCGCGG			370
	CAGGAGCTG GCGCAGGATG CCGCAGCGCT TCGGTGCGA GGGGTGCTTC TGAAGGAGTT			430
	CAGTGTGTC GCGACATAC TGACTATCGG ACTGACTGCT GCAGACCGCC GCGAAGTCCA			490
25	GCTCTCATC AGCTCTGTC TCCAGCAGT TTCCGTGTG ATGTGGATCA CCGAGTGTCT			550
	TCTGCGGTG TTTTGGCTC AGCTCTGTC AGGCGAGAG CCGTAAGGCC AGCTGGGCG			610
	CCCTCTAG GTGATGCTC CTCCCTAGG GAATGCTCC AGCAGGAGTG GCGAGTTCAT			670
30	TGTGGGGGCC TGATGTGTG TCGTGGAGG AGGAGCGCTT ACATGTTGT TTCTGTAGAA			730
	AATAAACTG AGCTAGAAA AA			752
35				

(2) INFORMATION FOR SEQ ID NO: 10:

- 40 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 58 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- 45 (ii) MOLECULE TYPE: protein

WO 00/23584

PCT/EP99/07832

13/18

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu Met Ala Gln Gly Ala
 1 5 10 15
 5 Met Leu Ala Ala Gln Glu Arg Arg Val Pro Arg Ala Ala Glu Val Pro
 20 25 30
 10 Gly Ala Gln Gly Gln Gln Gly Pro Arg Gly Arg Glu Glu Ala Pro Arg
 35 40 45
 Gly Val Arg Met Ala Ala Arg Leu Gln Gly *
 50 55

15

2) INFORMATION FOR SEQ ID NO: 11:

20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 11 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

30 Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu
 1 5 10....

35 2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 10 amino acids
 (B) TYPE: amino acid
 40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

45 Leu Met Ala Gln Glu Ala Leu Ala Phe Leu
 1 5 10.

50

(2) INFORMATION FOR SEQ ID NO: 13:

55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

SUBSTITUTE SHEET (RULE 26)

WO 00/23584

PCT/EP99/07832

14/18

(ii) MOLECULE TYPE: synthetic DNA

5 GGIGACACTA TAGAAGGTAC G 21

(2) INFORMATION FOR SEQ ID NO: 14:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic DNA

20 TGATGTGCAA CTGAAGCAGG.....20

25 (2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

30

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic DNA

35 GCACTGCGIG ATCCACATCA A 21

40 (2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

45

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic DNA

50 CGACTCACTA TAGGGAGAGA G 21

WO 00/23584

PCT/EP99/07832

15/18

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: synthetic DNA

GCACATCAG ATGCGTTTCT CGTGG

25

15

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: synthetic DNA

CACACAAGC TTGGCTTAGC GCGCTGGC TG.....32

30

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: synthetic DNA

CACACAGAT CCATGGATC TGCAGATGG.....30

45

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

- 50 (A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: synthetic DNA

GAAGACATA TGCTGATGC CCAGGAGC

29

SUBSTITUTE SHEET (RULE 26)

WO 00/23584

PCT/EP99/07832

16/18

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic DNA

TTAAGATCT CAGAACGGCC OCTGGTGG

28

15

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic DNA

25

ttactogaga tgctgatggc ccagg.....25

30

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic DNA

40

aaggtaacctt gaacggcccc tggtag26

45

2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

50

WO 00/23584

PCT/EP99/07832

17/18

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Phe Leu Met Ala Gln Gly Ala Met Leu
 1 5 9

5

2) INFORMATION FOR SEQ ID NO: 25:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Ala Met Leu Ala Ala Gln Glu Arg Arg Val
 1 5 10

25

2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

30

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Met Leu Ala Ala Gln Glu Arg Arg Val
 1 5 9

40

2) INFORMATION FOR SEQ ID NO: 27:

45

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Tyr Tyr Met Asn Gly Thr Met Ser Gln Val
 1 5 10

55

SUBSTITUTE SHEET (RULE 26)

WO 00/23584

PCT/EP99/07832

18/18

2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Glu Val Asp Pro Ile Gly His Leu Tyr
1 5 9

15

Claims

1. Tumor-associated antigen CAMEL which is encoded by the ORF-1 of LAGE-1 cDNA and has the amino acid sequence set forth in
5 SEQ ID NO: 2.
2. The tumor-associated antigen of claim 1 for use in cancer therapy.
3. Immunogenic (poly)peptide derived from the tumor-associated antigen of claim 1.
4. An immunogenic peptide of claim 3, characterized in that it has the
10 amino acid sequence Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu (SEQ ID NO: 11).
5. An immunogenic peptide of claim 3, characterized in that it has the amino acid sequence Leu Met Ala Gln Glu Ala Leu Ala Phe Leu (SEQ ID NO: 12).
- 15 6. An immunogenic peptide of claim 3, characterized in that it has the amino acid sequence Phe Leu Met Ala Gln Gly Ala Met Leu (SEQ ID NO: 24).
7. An immunogenic peptide of claim 3, characterized in that it has the amino acid sequence Ala Met Leu Ala Ala Gln Glu Arg Arg Val
20 (SEQ ID NO: 25).
8. An immunogenic peptide of claim 3, characterized in that it has the amino acid sequence Met Leu Ala Ala Gln Glu Arg Arg Val (SEQ ID NO: 26).
9. An immunogenic peptide of any one of claims 3 to 8 for use in cancer
25 immunotherapy.
10. Pharmaceutical composition containing the tumor-associated antigen CAMEL of claim 1.

WO 00/23584

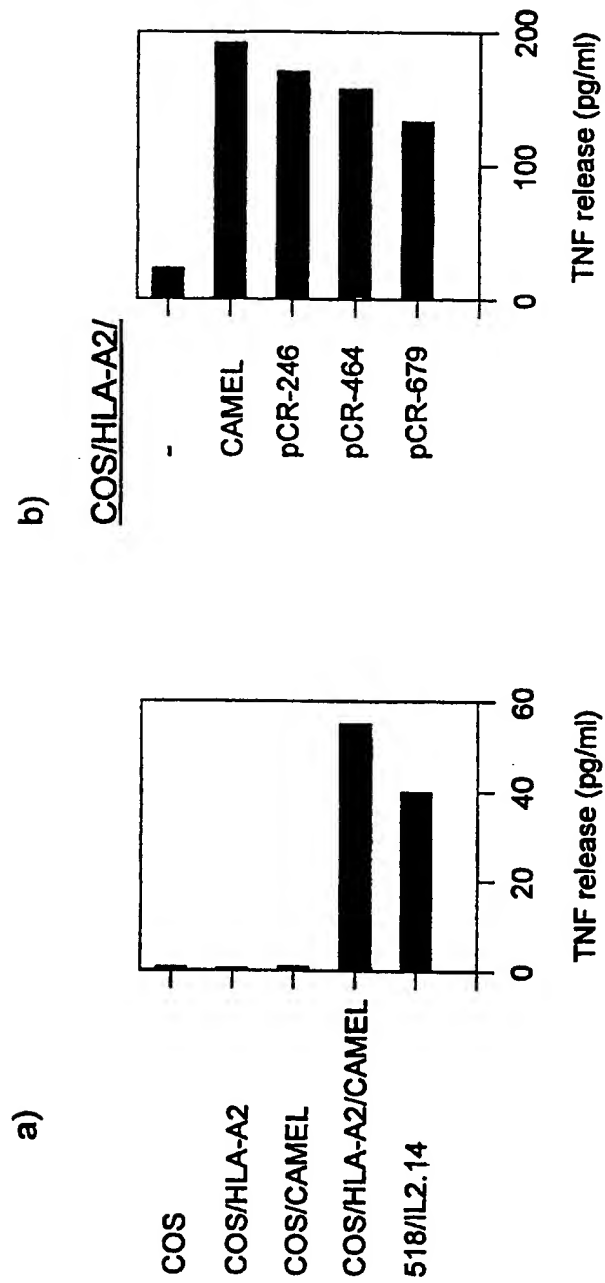
PCT/EP99/07832

35

11. Pharmaceutical composition containing an immunogenic peptide of any one of claims 3 to 8.
12. Isolated DNA molecule comprising the sequence set forth in SEQ ID NO: 1.
- 5 13. Recombinant DNA molecule comprising the DNA molecule of claim 12.
14. A DNA molecule of claim 12 or 13 for use in cancer immunotherapy.

1/15

Fig. 1



WO 00/23584

PCT/EP99/07832

2/15
Fig. 2A

FIGURE 2 A)

CAMEL	-----	
LAGE-1 ^s	--ATCCTCGTGGGCCCTGACCTTCTCTCTGAGAGCCGGGCAGAGGCTCCG	48
LAGE-1 ^L	GCATCCTCGTGGGCCCTGACCTTCTCTCTGAGAGCCGGGCAGAGGCTCCG	50
NY-ESO-1	--ATCCTCGTGGGCCCTGACCTTCTCTCTGAGAGCCGGGCAGAGGCTCCG	48
CAMEL	-----	
LAGE-1 ^s	GAGCC ATG CAGGCCGAGGCCAGGCCACAGGGGTTTCGACGGGCGATGCT	14
LAGE-1 ^L	GAGCC ATG CAGGCCGAGGCCAGGCCACAGGGGTTTCGACGGGCGATGCT	98
NY-ESO-1	GAGCC ATG CAGGCCGAGGCCAGGCCACAGGGGTTTCGACGGGCGATGCT	100
	*****	98
CAMEL	-----	
LAGE-1 ^s	GATGGCCCAGGAGGCCCTGGCATTCCCTGATGGCCCCAGGGGCAATGCTGG	64
LAGE-1 ^L	GATGGCCCAGGAGGCCCTGGCATTCCCTGATGGCCCCAGGGGCAATGCTGG	148
NY-ESO-1	GATGGCCCAGGAGGCCCTGGCATTCCCTGATGGCCCCAGGGGCAATGCTGG	150
	*****	148
CAMEL	-----	
LAGE-1 ^s	CGGCCCCAGGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG	114
LAGE-1 ^L	CGGCCCCAGGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG	198
NY-ESO-1	CGGCCCCAGGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG	200
	*****	198

WO 00/23584

PCT/EP99/07832

3/15

Fig. 2A continued

CAMEL	GGGCAGCAGGGCCTCGGGCCGAGAGGAGGCGCCCGGGGGTCCGCGAT	164
LAGE-1 ^s	GGGCAGCAGGGCCTCGGGCCGAGAGGAGGCGCCCGGGGGTCCGCGAT	248
LAGE-1 ^L	GGGCAGCAGGGCCTCGGGCCGAGAGGAGGCGCCCGGGGGTCCGCGAT	250
NY-ESO-1	GGGCAGCAGGGCCTCGGGCCGAGAGGAGGCGCCCGGGGGTCCGCGAT	248

CAMEL	GGCGGTGCCGCTTCTGCGCAGGATGGAAGTGCCCTGCGGGGCCAGGAG	214
LAGE-1 ^s	GGCGGTGCCGCTTCTGCGCAGGATGGAAGTGCCCTGCGGGGCCAGGAG	298
LAGE-1 ^L	GGCGGTGCCGCTTCTGCGCAGGATGGAAGTGCCCTGCGGGGCCAGGAG	300
NY-ESO-1	GGCGGTGCCGCTTCTGCGCAGGATGGAAGTGCCCTGCGGGGCCAGGAG	298

CAMEL	GCCGGACAGCCGCTTCAAGTTGCACATCACGATGCCTTCTCGTCGC	264
LAGE-1 ^s	GCCGGACAGCCGCTTCAAGTTGCACATCACGATGCCTTCTCGTCGC	348
LAGE-1 ^L	GCCGGACAGCCGCTTCAAGTTGCACATCACGATGCCTTCTCGTCGC	350
NY-ESO-1	GCCGGACAGCCGCTTCAAGTTGCACATCACGATGCCTTCTCGTCGC	348

CAMEL	CCATGGAAGCGGAGCTGGTCCGAGGATCCTGTCCCGGGATGCCGCACCT	314
LAGE-1 ^s	CCATGGAAGCGGAGCTGGTCCGAGGATCCTGTCCCGGGATGCCGCACCT	398
LAGE-1 ^L	CCATGGAAGCGGAGCTGGTCCGAGGATCCTGTCCCGGGATGCCGCACCT	400
NY-ESO-1	CCATGGAAGCGGAGCTGGTCCGAGGATCCTGTCCCGGGATGCCGCACCT	398

Fig. 2A continued

CAMEL	CTCCCCGACCAAGGGCGGTTCTGAAGGACTTCACCGTGTCCGGCAACCT	364
LAGE-1 ^s	CTCCCCGACCAAGGGCGGTTCTGAAGGACTTCACCGTGTCCGGCAACCT	448
LAGE-1 ^L	CTCCCCGACCAAGGGCGGTTCTGAAGGACTTCACCGTGTCCGGCAACCT	450
NY-ESO-1	CTTCCCGTGCCAGGGGTGCTTCTGAAGGAGTTCACTGTGTCCGGCAACAT	448
	** *** .. ***** * ***** * ***** * ***** *	
CAMEL	ACTGTTTAT-----	373
LAGE-1 ^s	ACTGTTTAT-----	457
LAGE-1 ^L	ACTGTTTATGTCAGTTCGGGACCAGGACAGGAGCGCTGGGCGGATGA	500
NY-ESO-1	ACTGACTAT-----	457
	****. ***	
CAMEL	-----	373
LAGE-1 ^s	-----	457
LAGE-1 ^L	GGTGGTGGGTTGGGGCTGGGATCCGCCTCCCGAGGGCAGAAAGCT	550
NY-ESO-1	-----	457
CAMEL	-----	373
LAGE-1 ^s	-----	457
LAGE-1 ^L	AGAGATCTCAGAACACCCAAACACAAGGTCTCAGAACAGAGACCTGGTAC	600
NY-ESO-1	-----	457

WO 00/23584

PCT/EP99/07832

5/15

Fig. 2A continued

CAMEL	373	-----
LAGE-1 ^s	457	-----
LAGE-1 ^L	650	ACCAGGCCCGCCGACCCGAGGAGCCAGGAGATGGGTGCAGAGGTG
NY-ESO-1	457	-----
CAMEL	385	-----CCGACTGACTGC
LAGE-1 ^s	469	-----CCGACTGACTGC
LAGE-1 ^L	700	TCGCCCTTAATGTGATGTTCTCTGCCCCCTCACATTTAGCCGACTGACTGC
NY-ESO-1	469	-----CCGACTGACTGC

CAMEL	435	TGCAGACCACCGCCAACTGCAGCTCTCCATCAGCTCCTGTCTCCAGCAGC
LAGE-1 ^s	519	TGCAGACCACCGCCAACTGCAGCTCTCCATCAGCTCCTGTCTCCAGCAGC
LAGE-1 ^L	750	TGCAGACCACCGCCAACTGCAGCTCTCCATCAGCTCCTGTCTCCAGCAGC
NY-ESO-1	519	TGCAGACCACCGCCAACTGCAGCTCTCCATCAGCTCCTGTCTCCAGCAGC

CAMEL	485	TTTCCCTGTTGATGTGGATCACGCAGTGCTTTCTGCCCGTGTTTTGGCT
LAGE-1 ^s	569	TTTCCCTGTTGATGTGGATCACGCAGTGCTTTCTGCCCGTGTTTTGGCT
LAGE-1 ^L	800	TTTCCCTGTTGATGTGGATCACGCAGTGCTTTCTGCCCGTGTTTTGGCT
NY-ESO-1	569	TTTCCCTGTTGATGTGGATCACGCAGTGCTTTCTGCCCGTGTTTTGGCT

Fig. 2A continued

CAMEL
LAGE-1^s
LAGE-1^L
NY-ESO-1

CAGGCTCCCTCAGGGCAGAGCGGCTAAGCCCAGCCCTGGCGCCCCCTTCCTA 535
CAGGCTCCCTCAGGGCAGAGCGGCTAAGCCCAGCCCTGGCGCCCCCTTCCTA 619
CAGGCTCCCTCAGGGCAGAGCGGCTAAGCCCAGCCCTGGCGCCCCCTTCCTA 850
CAGGCTCCCTCAGGGCAGAGCGGCTAAGCCCAGCCCTGGCGCCCCCTTCCTA 519
*** *****

CAMEL
LAGE-1^s
LAGE-1^L
NY-ESO-1

GGTCATGCCCTCCTCCCTAGGGAATGGTCCCAGCACGAGTGGCCAGTTCA 585
GGTCATGCCCTCCTCCCTAGGGAATGGTCCCAGCACGAGTGGCCAGTTCA 669
GGTCATGCCCTCCTCCCTAGGGAATGGTCCCAGCACGAGTGGCCAGTTCA 900
GGTCATGCCCTCCTCCCTAGGGAATGGTCCCAGCACGAGTGGCCAGTTCA 669

CAMEL
LAGE-1^s
LAGE-1^L
NY-ESO-1

TTGTGGGGCCCTGATTGTTGTCGCTGGAGGAGGACGGCTTACATGTTG 635
TTGTGGGGCCCTGATTGTTGTCGCTGGAGGAGGACGGCTTACATGTTG 719
TTGTGGGGCCCTGATTGTTGTCGCTGGAGGAGGACGGCTTACATGTTG 950
TTGTGGGGCCCTGATTGTTGTCGCTGGAGGAGGACGGCTTACATGTTG 719

CAMEL
LAGE-1^s
LAGE-1^L
NY-ESO-1

TTTCTGTAGAAAATAAAGCTGAGCTACGAAAAAATAAAAAA 679
TTTCTGTAGAAAATAAAGCTGAGCTACGAAAAAATAAAAAA 767
TTTCTGTAGAAAATAAAGCTGAGCTACGAAAAAATAAAAAA 993
TTTCTGTAGAAAATAAAGCTGAGCTACGAAAAAATAAAAAA 752

7/15

Fig. 2B

Protein Translations

ORF3

LAGE-1^S MQAEGQGTGGSTGDADGPGGPGIPDGPGGNAGGPGGEAGAT 40
 LAGE-1^L MQAEGQGTGGSTGDADGPGGPGIPDGPGGNAGGPGGEAGAT 40
 NY-ESO-1 MQAEGRGTGGSTGDADGPGGPGIPDGPGGNAGGPGGEAGAT 40

LAGE-1^S GGRGPRGAGAAARASGPRGGAPRGPHGGAASAQDGRCP CGA 80
 LAGE-1^L GGRGPRGAGAAARASGPRGGAPRGPHGGAASAQDGRCP CGA 80
 NY-ESO-1 GGRGPRGAGAAARASGPGGGAPRGPHGGAASGLNGCCRC CGA 80

LAGE-1^S RRPDSRLLQLHITMPFSSPMEAEELVRRILSRDAAPLPRPG 120
 LAGE-1^L RRPDSRLLQLHITMPFSSPMEAEELVRRILSRDAAPLPRPG 120
 NY-ESO-1 RGPESRLLLEFFYLAMPFATPMEAEELARRSLAQDAPPLPVPG 120

LAGE-1^S AVLKDFTVSGNLLFIRLTAADHRQLQLSISSCLQQLSLLM 160
 LAGE-1^L AVLKDFTVSGNLLFM~~SV~~RDQDREGAGRM~~RV~~VGWGLGSASP 160
 NY-ESO-1 VLLKEFTVSGNILLTIRLTAADHRQLQLSISSCLQQLSLLM 160

WO 00/23584

PCT/EP99/07832

8/15

Fig. 2B continued

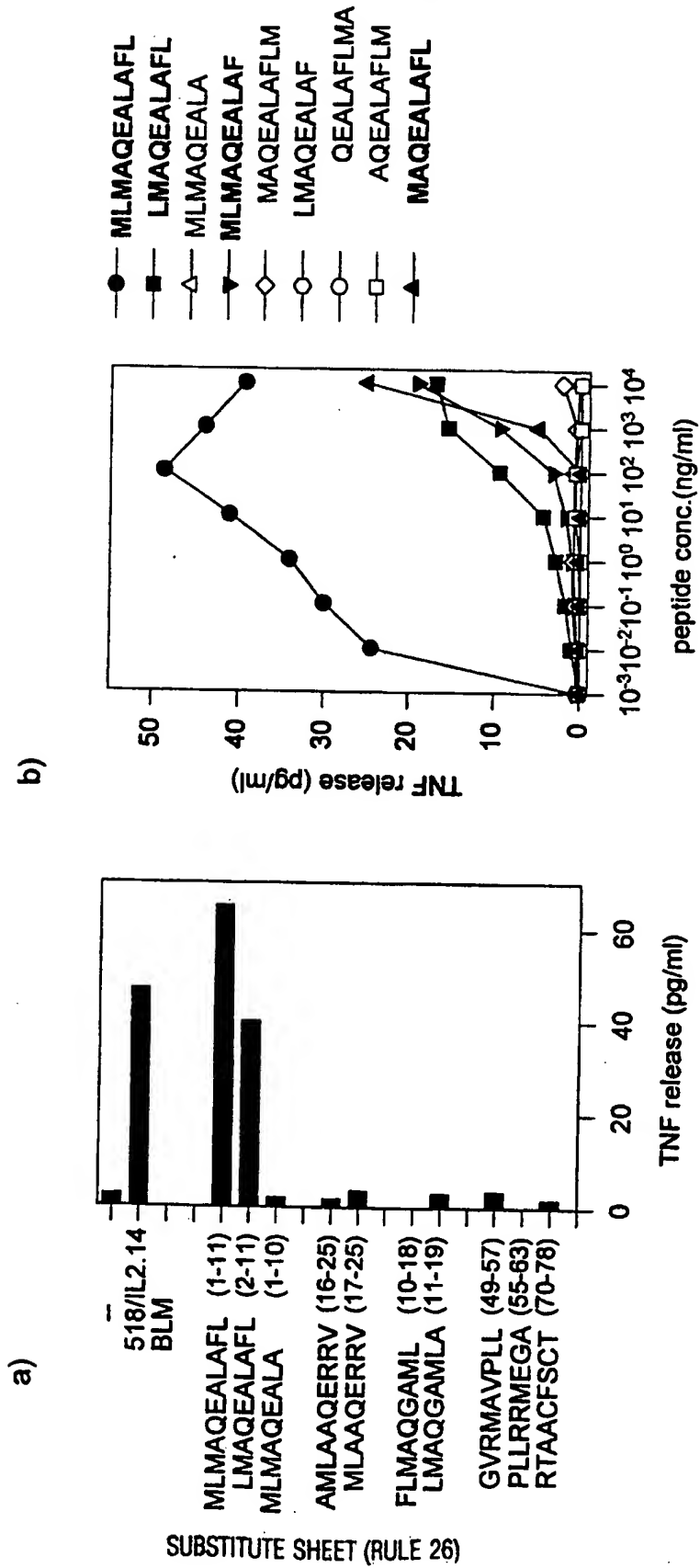
LAGE-1 ^s	WITQCFLPVFLAQAPSGQRR	180
LAGE-1 ^L	EGQKARDLRTPKHKVSEQRPGTGPPEGAQGDGCRGVA	200
NY-ESO-1	WITQCFLPVFLAQPPSGQRR	180
LAGE-1 ^s		180 aa, 18.2 kD
LAGE-1 ^L	FNVMFSAPHI	210 aa, 21.1 kD
NY-ESO-1		180 aa, 18.2 kD
ORF1		
LAGE-1 ^s	<u>MLMAQEALAF</u> MLMAQGAMLAQAQERRVPRAAEVPGAQQQGP	40
LAGE-1 ^L	<u>MLMAQEALAF</u> MLMAQGAMLAQAQERRVPRAAEVPGAQQQGP	40
NY-ESO-1	<u>MLMAQEALAF</u> MLMAQGAMLAQAQERRVPRAAEVPGAQQQGP	40
LAGE-1 ^s	RGREEAPRGVRMAVPLLLRRMEGAPAGPGGRTAACFSCTSR	80
LAGE-1 ^L	RGREEAPRGVRMAVPLLLRRMEGAPAGPGGRTAACFSCTSR	80
NY-ESO-1	RGREEAPRGVRMA ARLQG	58
LAGE-1 ^s	CLSRRPWKRWSAGSCPGMPHLSPDQGRF	109 aa, 11.7 kD
LAGE-1 ^L	CLSRRPWKRWSAGSCPGMPHLSPDQGRF	109 aa, 11.7 kD
NY-ESO-1		58 aa, 6.2 kD

WO 00/23584

PCT/EP99/07832

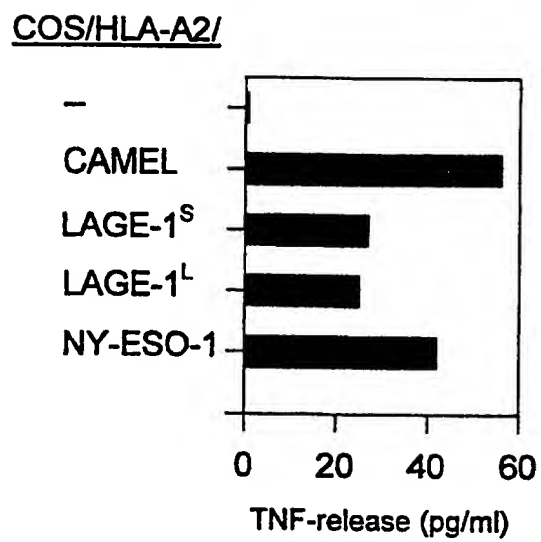
9/15

Fig. 3



10/15

Fig. 4

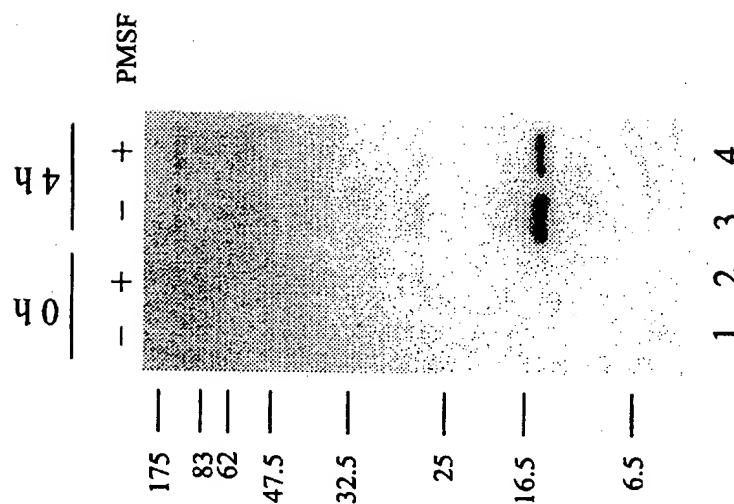


WO 00/23584

PCT/EP99/07832

11/15

Fig. 5

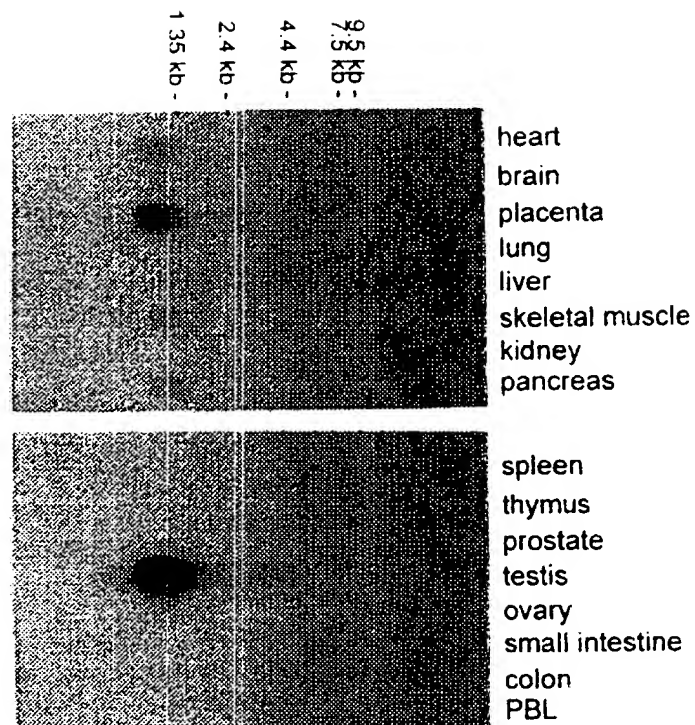


WO 00/23584

PCT/EP99/07832

12/15

Fig. 6A



13/15

Fig. 6B

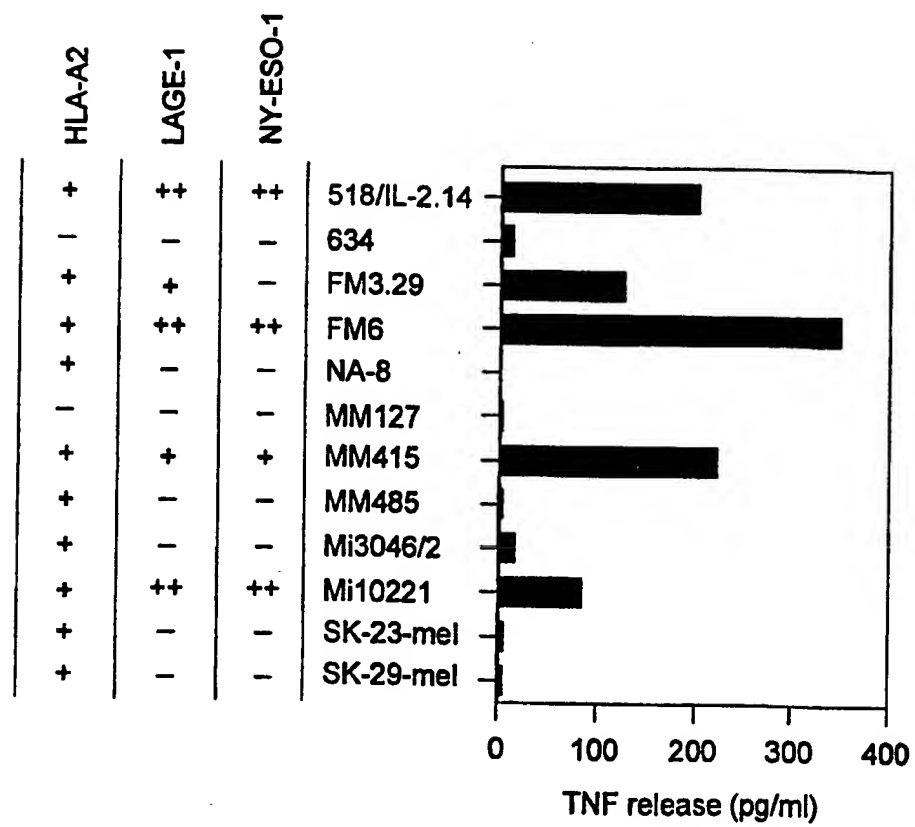
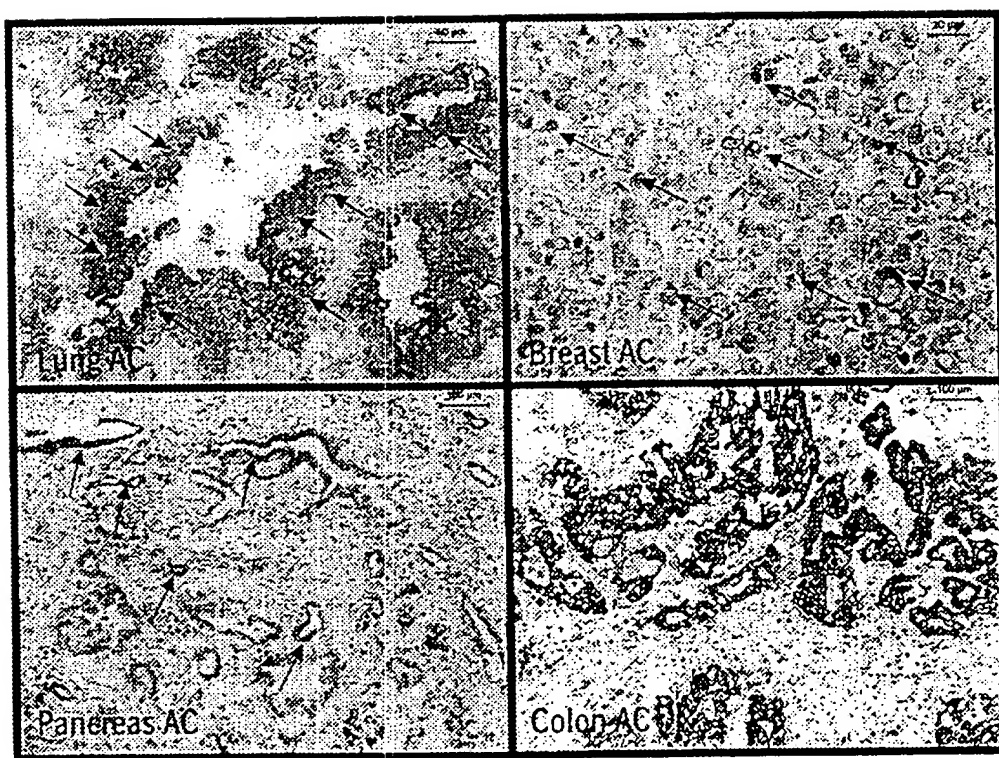


Fig. 7



WO 00/23584

PCT/EP99/07832

15/15

Fig. 8

